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ALLEN JOHN SMITH—A TRIBUTE

Allen John Smith was born at York, Pa., Dec. 8, 1863, the son of Gibson and Susan Elizabeth Fahs Smith, both descendants of prosperous merchants and farmers of the "Country Dutch" of Pennsylvania. He received his preparatory education at the York Academy and attended Gettysburg College from which he received the degree of A.B. in 1883. He studied medicine at the University of Pennsylvania, receiving his M.D. in 1886. Later he was the recipient of other degrees: Sc.D. from Gettysburg in 1910, LL.D. from McGill University in 1911 and again from Gettysburg College in 1921.

After serving a year as Resident Physician at the Philadelphia General Hospital ("Blockley"), he decided that pathology was a subject of vital importance to which he would devote at least a part of his life, and connected himself with the Medical Department of the University of Pennsylvania as an Assistant Demonstrator under Dr. John Guiteras, the professor, and Dr. Henry Formad, the demonstrator of Pathology. In this small position—it then paid a salary of only *one hundred dollars a year*—he so distinguished himself for industry, energy and capacity, that three years later he was recommended for the professorship of pathology in the University of Texas, to which he was elected in 1891.

The work that he did in Texas was of epoch-making importance to the Medical Department of its University, for with small means, a faculty of only eight or ten to begin with and very limited facilities, under his direction, as Dean, in ten years it became a worthy school. Only his colleagues really know and can tell of that part of his life, but to some of his close friends in the north he now and then seriously or facetiously confided an occasional incident. For example, there being but a few microscopes, Smith became "professor of all subjects taught with the aid of microscopes"—normal histology, embryology, pathological histology, bacteriology, parasitology, clinical pathology, etc. There being but few faculty members, he held himself ready to substitute for any one ill or temporaily absent. He devoted himself for a time to nervous diseases and lectured upon them; to clinical medicine at another time and unintentionally acquired a consulting practice.

He returned to the University of Pennsylvania to be its professor of pathology in 1903, but during the twenty-three years that followed, his recollections of the University of Texas were most fond and happy. He was proud of his record there, frequently longed to return and not

one of his intimates would have been surprised had he done so. At the University of Pennsylvania he continued as professor of pathology until 1912, adding to his duties the office of Dean of the Faculty from 1909 to 1912. In the latter year, through a reorganization of the Faculty, he was made the head of a new Department of Comparative Pathology and Tropical Medicine, and ceased to be dean. But in 1913 he again became professor of Pathology, which position he held until his death on August 18, 1926. During the World War, when so many were called away to the service of their country, he again acted as Dean of the Faculty until he was himself called to active service, first at Camp Dix and later at Camp Pike. Entering with the rank of Major, he retired from active service a Lieutenant Colonel in the Medical Reserve Corps of the United States Army, and left behind him a record for faithful and disinterested service.

Smith had a remarkable faculty for making people his friends. Without soliciting it, and no doubt in many cases contrary to his intentions, he became the custodian of confidences that might have led a less tactful man into institutional intrigues. He was above such things, and though his high sense of honor and justice frequently made him revolt at the actions of others, he was not one to either pick a quarrel or to harbor resentment. The soundness of his judgment and the generosity with which he lent it to others, together with his genial disposition and affability, led him unwittingly to encourage those of any station or position, so that it inevitably happened that all of his time was claimed by others and none left for himself.

His primary and dominant interest was the medical student, and teaching was his delight. He was too willing to confide in others. He thus became the victim of his friends. It was through such a circumstance that the most distinguished of his works, the discovery of the American hookworm, has been undeservedly attributed to another. Shyness kept him aloof from the throng of men. He preferred the retirement of his library and laboratory to attendance upon meetings. He industriously collected specimens—especially parasitological material—and notes, with the intention of publishing, but too often postponed this, awaiting more complete information or until a greater series of specimens should be assembled or a more convenient time was at hand.

He arrived at his rooms at the University before 9 a. m., and remained there until 5 p. m. not going out for lunch, but eating a sandwich while sitting at his desk. All days, working days and holidays, were alike to him, he was busy all of the time—mostly with the affairs of students and alumni—and he took no vacation in 27 years! No man will be more missed than he, for he gave himself to his friends. But notwithstanding these distractions he succeeded in doing a considerable

amount of telling work. In a summary of the activities of the various members of the staff of the department of pathology—past and present—of the University of Pennsylvania, he mentions and evaluates his own work in these words:

1. Class-book in Bacteriology (this was a synopsis prepared for students).

2. Text-book of Comparative General Pathology, by Dr. Th. Kitt, translated from the German by William W. Cadbury and Allen J. Smith, Chicago, W. T. Keener & Co., 1906. (Here the assistant, Dr. Cadbury, is generously given first place.)

3. Chemistry of Metabolism by Otto von Fürth. Translated from the German by Allen J. Smith, Phila. J. B. Lippincott Co., 1916.

4. Sections in various text-books upon legal medicine, on various modes of deaths, animal parasites and the principles of immunity, and journal articles upon a variety of subjects in pathological anatomy, animal parasitology and tropical affections.

5. Discovery of *Bacillus coeruleus* (*Pseudomonas smithii*); establishment of hook-worm disease as endemic in the United States; production of evidence implicating bedbugs as conveyors of leprosy infection and of ticks as harboring larvae of certain filarial worms; establishment of a number of species of animal parasites; elucidation of periodicity of microfilaria in the peripheral blood of the hosts; production of eosinophilic granulations in leucocytes extracorporeally.

6. Rightly or wrongly as others and time must decide, the writer has held that the first duty of the department in a teaching institution is to teach the classes and has not hesitated to accept as appropriate, a large volume of autopsy and operative material for gross and minute investigation. Records of the latter have been kept and constitute evidence of the amount of work done by the pathological staff. The writer has held that such work is really worthy of recognition as investigation and is a necessary part of the duties of the department. Research has been favored for all members of the staff and others within the time left available after such work has been covered; and always time has been sought for individuals to make research possible for them.

He estimates his own work in these words:

"My own work upon hook-worm was fundamental and conclusive of the proposition that this affection was endemic in this country. It was largely overshadowed by the subsequent work of Stiles in establishing the fact that the particular species involved in this country was a new species, and by the vigorous campaign he was able to launch, largely through the Rockefeller Health Board, against this affection, conducive of inestimable benefit both to our own Southern States and to many other tropical and sub-tropical lands."

"In 1914 the writer (in collaboration with Dr. M. T. Barrett) brought forward again the virtually forgotten mouth amoeba (originally described by the Russian Gros, in 1849) and attributing to it an important part in producing the exceedingly common pyorrhoea alveolaris, with the further proposal to treat the affection by emetine as an amoebicide. The writer's position has frequently been assailed, mostly under the mistaken belief that the amoeba had been heralded as the sole cause of the affection; others have accepted the ideas proposed that the amoeba in a more or less symbiotic relation with a variety of bacteria, is causa-

tive. Whatever may be the ultimate decision, the work has unquestionably been stimulative to greater care in the study of mouth infections by both medical and dental professions."

One of the most careful appraisals of Smith's work upon hookworms is to be found in the monograph upon "Hookworm Disease" by George Dock and Charles C. Bass, St. Louis, Mo., C. V. Mosby Company, 1910. After an historical account from early times to 1905, they write as follows:

> The era of productive discovery began at this time; Allen J. Smith, in 1895, found ova of hookworm in the feces in a water closet in Galveston, Texas, but could not fix upon the individual from whom they were derived. On February 17, 1901, Dr. Smith recognized ova in the stools of a sailor born in Melbourne, Australia, who first became ill while acting as overseer of a plantation in southern Mexico, where there was an epidemic disease of very fatal type, which led to pallor, emaciation and dropsy.——Dr. Smith recognized differences in the worms expelled from the patient as compared with *Ankylostoma duodenale*, and thought they might be *Uncinaria stenocephala* of dogs.——Later Charles Wardell Stiles showed the existence of a new species in man, and Dr. Smith was able to demonstrate that his patient had both old world and new world parasites. Stiles had for years pointed out the probability of the frequent occurrence of hookworm in America. In 1901 he published his views—stating that hookworm in man must be more or less widespread in the United States. Soon after that Smith's case was published by Dr. Charlotte Shaeffer, who also mentioned the finding of ova in the feces of two (later increased to eight) out of eighty-odd medical students in the Galveston School. Stiles (1903) shows clearly the great value of Smith's work in the development of our knowledge of hookworm in the United States. "To the clinician it did not mean much, since no record existed that the students exhibited any very severe symptoms. To the zoologist, however, it meant a practical demonstration that uncinariasis was more or less common in the south. . . ."

With the death of Dr. Smith there passes away a teacher of the highest and most idealistic type, an indefatigable worker for the advancement of medical science and a kind and generous friend.

By the Staff of the Department of Pathology
of the University of Pennsylvania

Dr. Allen J. Smith was greatly interested in the JOURNAL OF PARASITOLOGY. When the idea of starting such a publication was first broached, he wrote commending it enthusiastically. He became a member of the original Editorial Board and in the early years was one of the few financial supporters of the enterprise. He also gave time and thought to its problems, writing promptly and fully in reply to the general letters sent the members of the Editorial Board for advice and assistance in meeting the difficulties which arose. He contributed valuable papers to the first and second volumes of the new periodical and later secured others from colleagues and friends. In fact he was constantly active with encouragement and stimulation and in securing

support of many sorts. It is a privilege to place on record here these facts, the significance of which I have recognized so clearly and appreciated so highly but which from the quiet manner in which they were done have naturally not come to the attention of others.

HENRY B. WARD.

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EGG-WORM CORRELATIONS IN CASES OF *FASCIOLOPSIS BUSKI*

WITH ADDITIONAL DATA ON THE DISTRIBUTION OF THIS PARASITE IN CHINA *

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The use of egg counts to secure information on the severity of hookworm infestations, has naturally raised the query as to their applicability to other worm parasites. While making examinations for hookworm at Shaohsing, China, opportunity was afforded to study a few individuals with *Fasciolopsis buski*. A brief report is made here of these findings, together with an analysis of egg counts of the China Hookworm Commission as far as they give information on the distribution of this intestinal fluke.

The typical dilution method as detailed by Cort, Grant and Stoll (1926) was used in securing the counts. Data on the intensively studied cases were obtained chiefly by one of us (Kwei) during May, 1924, at the Christian Hospital in Shaohsing. Previous to treatment egg counts were made as far as possible on all stools passed for three or more days. After weighing, each stool was well stirred before sampling for the counts. The treatments were under the direction of Dr. C. H. Barlow, whose cordial co-operation made this study possible. Beta-napthol was the drug used, with adult dose of 25 gr. given three times, an hour apart. The treatment was preceded the night before and followed an hour afterward by a magnesium sulphate purge. Stool collections for worm counts were made for three or more days, following which additional stools were saved for post-treatment egg counts. On some of the cases second treatments and worm counts were secured in the same way.

RESULTS WITH INTENSIVELY STUDIED CASES

It was found even easier to make dilution counts of the *Fasciolopsis buski* ova than of hookworm. The eggs of the former are large and show up well on the slides, thus making them difficult to miss. Certain of our data are suggestive that the Fasciolopsis counts are more uniform

* Contribution from the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University. The data were gathered during the work of the China Hookworm Commission, which was financed by the International Health Board of the Rockefeller Foundation.

from slide to slide out of the same tube, and from tube to tube out of the same stool, than has been demonstrated for hookworm. The facility with which the counts are made was reflected in certain incidence data secured in Soochow, which will be given later in this article. The worm counts for *Fasciolopsis buski* are relatively simple, with such large parasites, and undoubtedly are more accurate, generally speaking, than for hookworm.

In all, eight cases were under study for periods of from 9 to 19 days. Table 1 brings together the eggs per day data for 6 of these. Cases G and H are omitted because there was loss of stools during the worm counts. This was especially unfortunate in Case H which had the highest single day's egg output in the series, namely 9,048,000, and was completely negative after one treatment. Cases A-D inc. had light Trichuris infections (less than 500 eggs per gram) and Cases D and E showed about 3,000 and 7,000 Ascaris eggs per gram respectively.

TABLE 1.—*Eggs Per Day in Fasciolopsis buski Cases Studied by Dilution Egg count*

Case	Age	Sex	Total Days Under Study	Before Treatment: Days Studied	Before Treatment: Av. Eggs per Day	After Treatment: Days Re-studied	After Treatment: Av. Eggs per Day	Worms Secured	Net Eggs per Day for Worms Secured	Av. Eggs per Day per Fluke
A	27	F	19	2	4,433,000	3	46,000	300*	4,387,000	14,623
B	18	F	19	4	6,054,000	1	133,000	256*	5,921,000	23,129
C	31	M	9	3	3,569,000	3	415,000	126	3,154,000	25,032
D	34	F	14	4	1,540,000	6	Negative	32	1,540,000	48,125
E	4	F	17	3	1,314,000	2	880,000	25	434,000	17,360
F	2	F	17	3	827,000	..	.....	20*	827,000	41,350
Total.....					17,737,000		1,474,000	759	16,263,000	

* Total for two treatments.

Case F had Fasciolopsis only. It is improbable that the Trichuris and Ascaris infections influence the Fasciolopsis data from the standpoint from which we considered them.

It will be noted that Cases A-E were egg counted from 2 to 4 days before, and from 1 to 6 days after treatment. Case F left the hospital before post-treatment egg counts could be secured, but is assumed to have become negative. These cases showed initial average egg outputs per day from 827,000 to 6,054,000, and worm counts from 20 to 300. They are significantly in the same order in both arrays, except that Cases A and B interchange position. This indicates at once a presumptive correlation between the two series although they are too short to demonstrate a valid biometric measurement. As the rule holds, then, that the egg counts per day and the number of flukes harbored rise and fall together, it is possible to examine the data to determine what "factor" in eggs per day is indicative of the number of flukes present. This may be attempted from these data from three viewpoints:

1. The average egg output per day after treatment may be subtracted from the like data before treatment, and the result divided by

the total number of flukes recovered. This has been done in the last column in Table 1, which shows a range of 14,623 to 48,125, with a mean of 28,269 eggs per day per fluke in these individuals. It will be noted that two of these cases, D and F, show considerably larger counts than the others. Their influence on the average rather indicates that the 28,269 mean is high.

2. It happens that inter-treatment egg counts were secured on Cases A and B, which also expelled the largest number of worms. If the number of eggs being passed after each treatment is subtracted from those passed just before, each of these cases furnish two other "eggs per day per fluke" estimates. For Case A, expelling 275 worms after first treatment this became 15,333; expelling 25 worms after second treatment this was 9920 (for the two treatments considered as in Table 1, it was 14,623). Similarly in Case B, first treatment expelled 151 worms averaging 26,252 eggs per day per fluke; second treatment expelled 105 worms averaging 18,635 (for the two treatments considered as in Table 1 it was 23,129). The rough similarity of these paired figures would indicate that even three or four days after beta-napthol treatment the remaining flukes have practically re-established their previous rate of oviposition. If the eggs per day per fluke "factors" are figured on the individual treatments of Cases A and B as just noted, the range would be from 9920 to 48,125, with an average for 8 "cases" of 25,251.

3. Finally, the computation may be made, not on the individual case, but on the individual worm as the centering point. Thus Cases A-F inclusive passed an average of 17,737,000 eggs per day before treatment, and 1,474,000 afterward. The net number of ova for the total of 759 flukes recovered from these cases was therefore, 16,263,000 per day, or 21,427 eggs per day per fluke. In so far as our data are adequate to the problem the true mean figure may be taken as lying somewhere between about 21,000 and 28,000, eggs per day per worm in *Fasciolopsis buski* cases, the intermediate figure (under 2 above) of about 25,000 probably striking close to a usable estimate.

It is true, of course, that if this figure were a constant from case to case, or worm to worm (regardless of size or age of the flukes), and all significant errors of technic were absolutely excluded, the estimate would be the same by the three methods just outlined. That they fall so relatively close together is indirect evidence that the approximate 25,000 figure has some validity. From the standpoint of the egg counts themselves it may be assumed that the data are relatively reliable, in that, as noted, dilution counts of *Fasciolopsis buski* ova are rather easy to make. As much is true of the worm counts. The greatest variability may be assumed to be due to the range in size and age of the flukes themselves.

In general these figures are somewhat higher, but overlap, a computation by Barlow (1925:8) on "a patient known to harbor but one fluke (for which) from ten to fifteen thousand ova per stool were estimated." Other studies on the egg outputs of intestinal flukes are apparently not extant. It is, perhaps, of interest that our figures for *Fasciolopsis buski* are somewhat comparable with the entirely unrelated hookworm correlations made in China, being in the range of those determined for mixed infections of *Ancylostoma duodenale* and *Necator americanus* encountered in the Soochow region (Cort, Grant and Stoll, 1926).

As in the hookworm studies (Stoll, 1923), the egg output per worm per day is the most fundamental and reliable relationship to be established between eggs in the stool and worms in the host. The usual egg count data secured from patients, however, are egg counts per gram, made on a random fecal sample from a stool. This is due to the fact that securing an isolated fecal specimen from an individual, even though he be a hospital in-patient, is much simpler than securing total stools covering several days in succession. The question, therefore, arises as to what relation if any, the egg counts per gram of stool, formed basis bear to the worms harbored.

The cases studied had come to the hospital primarily for fasciolopsiasis, in which as is well known "diarrhoea is a common symptom" (Barlow, 1925). So many loose stools were examined in our series, that a sufficiently accurate determination of a factor in terms of "eggs per gram, basis formed, per fluke" was unavailable. There is an indirect method of computing it by dividing the average egg output per day per fluke by the mean weight of formed stools. This, as given by Cort, Grant and Stoll (1926), is approximately 100 grams per day for adult Chinese. So determined, there would be a factor of between 210 and 280 eggs per gram per worm. It is possible that a figure near 200 is most reliable, inasmuch as the mean output per day per fluke for two of our cases (D and F) tend to raise the figure considerably over that of Cases A, B, C and E. To arbitrarily use 200 as the factor would appear therefore to be the best approximation within the limits of our present series, and due to the ease of interpolation would furnish a hypothetical worm burden from the eggs per gram counts which strikes somewhere near the true number. Such a figure must, at best, be considered usable only in relation to group egg counts, not to single individuals.

It was hoped, when this study was started, that a larger series might become available for analysis, and certain details in regard to symptoms, other laboratory findings, etc., were made. Too few cases are involved to make the data useful, however, no correlation being demonstrable in the hemoglobin or blood cell numbers or ratios, or in any objectively measured symptoms, with either the egg or worm counts.

INCIDENCE DATA

During the course of the work of the China Hookworm Commission at the Soochow Hospital, Kiangsu Province, a study was made of the comparative value of the dilution egg counting method and the simple smear in revealing positive cases of the various intestinal helminths. In examinations on 435 cases the Hospital laboratory, using the smear method, found 2 individuals or 0.4 per cent. positive for Fasciolopsis; the egg count demonstrated 8 or 1.8 per cent. positive. One case was found positive by both methods and had 5100 eggs per gram. The other seven positive by egg count (but missed by smear) showed respectively 30, 30, 50, 50, 100, 250, and 270 eggs per gram (not "formed basis"). This would indicate that the dilution count was considerably more accurate in detecting light Fasciolopsis cases than the smear method, although the comment is warranted in regard to the "dilution

TABLE 2.—*Incidence Data by Dilution Egg Counts of Fasciolopsis buski from Examinations of China Hookworm Commission*

Locality	Persons Examined	Number Eggs Counted	Number Positive for Fasciolopsis buski	Per Cent Postive
Chefoo, Shantung.....	City dwellers.....	125	None	0
Chefoo, Shantung.....	Villagers and farmers.....	890	2	0.2
Wuchang, Hupeh.....	In-patients and farmers....	562	4	0.7
Nantungchow, Ku.....	Cotton farmers.....	706	None	0
Soochow, Ku.....	Hospital in-patients.....	618	12	1.9
Soochow, Ku.....	Students.....	873	43	4.9
Soochow, Ku.....	Mulberry farmers.....	770	16	2.1
Soochow, Ku.....	Rice farmers.....	615	6	1.0
Canton, Ku.....	Farmers and villagers.....	851	5	0.6
Total.....		6009	88	

positives—smear negatives" that they are minimal sized infections, probably of one worm each. While the missing of such cases might lead to no particular harm to the patients, their "negative" status would tend to obscure endemic foci of the parasite, if they were being sought. Salt concentration methods, so useful in diagnosing intestinal nematodes, are of course, of little or no value with the trematode ova. It is thus obvious that the dilution count has a place with straight centrifugation in revealing Fasciolopsis cases missed by smear.

In Table 2 we have brought together the incidence data on *Fasciolopsis buski* as found in the examinations made by the Hookworm Commission in several localities in China. The total number of positive cases is small, being 88 out of 6009 examinations, or less than 2 per cent. Certain additional facts concerning these cases are of considerable interest however. Of the 1015 egg counts made in the Chefoo region, only two were positive, but these were both village children, boys aged 3 and 15 respectively. While their egg counts were low (50 and 350 eggs, per gram, basis formed) the fact of their youth may mean that they give evidence of a hitherto unsuspected endemic center of this

parasite in northern Shangtung province. In the Wuchang region, Hupeh province, 4 in 562 individuals showed infection with this fluke, one of them being a farmer boy of 11 years. The other three cases were in adults, and the four cases all had 400 eggs per gram or less. Wassell in 1924 reported an undoubted endemic case of *Fasciolopsis buski* from Hupeh. Of 705 individuals examined from Nantungchow, Kiangsu, just north of the Yangtze river, none showed Fasciolopsis ova, and in the Canton region, of 851 persons egg counted only 5 were found with ova (exact egg counts not being made on these cases).

The situation in the Soochow examinations was more striking, and evidently points to endemic centers of this parasite in this southern portion of Kiangsu province, which is just across Hangchow Bay from the important Shaohsing center. Thus in the mulberry area studied between Wusih and Soochow, 16 of 770 individuals showed Fasciolopsis ova. Of these 10 were males, and 6 females, with ages from 12 to 59 years. All the egg counts were 500 eggs per gram or less, formed basis, except a male 17 with 4800 eggs per gram. Among the rice farmers, of whom 615 were examined from villages about ten miles from Soochow, 6 were positive. Here again the egg counts were low, all 200 per gram or less except one male, age 27, with 78,000 eggs per gram. Inquiry showed that this man had originally come from the Shaohsing region. Of the Soochow Hospital in-patients, 12 of 618 examined showed Fasciolopsis ova, all having 500 or less eggs per gram, basis formed, except a merchant, age 23 with 5100. This man, and one other, were from Changshu, Ku., a fact of probable significance in pointing to an endemic center of the parasite.

In a student survey made in Soochow, 43 individuals, all males, out of 873 showed Fasciolopsis ova. This, an incidence of 4.9 per cent., was the highest encountered in any of the groups studied. The majority of these, 24, had no other worm parasites. While most of the counts were less and 1000 eggs per gram, basis formed, 8 were higher with 1100 1600, 1700, 1800, 3700, 38,000, and 7300 eggs per gram respectively. Figured in terms of group amount of infection, there was an average of 833 eggs per gram for all positive, and 41 eggs per gram for all examined. (This same student group showed a mean hookworm egg count of 19 eggs per gram.) Home addresses of 18 of the positive cases were known, and 6 of these were from Changshu, an item of special interest in view of the high egg count case in the Hospital series from this town.

In view of the fact that students are fond of buying water chestnuts "on the street," too much significance cannot be implied from these figures, as to the exact source of the infection. When it is considered, however, that 24 out of 43 students with *Fasciolopsis buski* had no other worm parasites, that the student group showed the highest incidence of any of the people that came within range of our study outside

the Shaohsing center itself, and that included in these are at least a few cases with Fasciolopsis worm burdens probably large enough to do clinical damage, there is evidently a field of hygienic instruction open to teachers in colleges and middle schools in this part of China which ought not be overlooked. In this connection the words of Barlow (1925) may be recalled: "It is not likely that a patient harboring over ten flukes would be without noticeable symptoms, and many a patient, with but one or two, often shows appreciable disease."

SUMMARY AND CONCLUSIONS

1. In a series of six intensively studied individuals harboring *Fasciolopsis buski,* an egg output per day per fluke was determined, that ranged from 14,623 to 48,125 ova. The mean output per fluke per day appears, from these cases, to lie between about 21,000 and 28,000 ova.

2. While our series is too small to determine the eggs per gram per fluke factor, a figure about one-hundredth of the eggs per day is presumably a close approximation.

3. It is demonstrated that the dilution egg counting method is superior to the smear in determining the incidence of positive cases with this parasite.

4. A resume of 6009 examinations made in various parts of China revealed 88 positives, chiefly in that part of southern Kiangsu province not far distant from the endemic areas of fasciolopsiasis in northern Chekiang.

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TRIGONOMONAS DIPLOSTOMUM, N. SP. FROM THE INTESTINE OF THE FROG

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In the course of some observations on intestinal parasites of the frog, *Rana clamitans,* a flagellated organism was found in a considerable proportion of the freshly killed frogs. As I though this organism to be of some morphological interest, and as it was of common occurrence in the intestinal contents of different lots of frogs, a study of its morphology was undertaken. About thirty per cent of the frogs examined showed a mild infection with the flagellate in question. Fresh and stained preparations were made from the intestinal contents, but the organisms not being numerous, it was necessary to cultivate them in order to obtain material sufficiently rich for morphological study. Generous samples of the intestinal contents, which contained many Trichomonas and Hexamitus, as well as a few of the flagellates to be studied, were placed in different culture media. The flagellates grew in large numbers in a medium made of N.N.N. agar base slants, containing potassium phosphate as a buffer, to which was added about 3 cc. of physiologic salt solution containing 0.5 per cent of "Difco," Loeffler's dried serum. Good growth was observed after four days incubation at room temperature. Numerous Trichomonas and Hexamitus were present in all the tubes, but no attempt was made to isolate the flagellate under study from these other organisms.

The flagellate was studied in unstained fresh preparations, under a coverslip which was sealed to the slide with paraffin; and also by using vital stains, which lessened the activity of its movements and stained some of its structures. Thin films of weak alcoholic solutions or neutral red and janus green were made on perfectly clean slides and allowed to dry. A drop of the culture containing the flagellates was placed on the dried film of the dye, then covered with a coverslip and sealed with paraffin. Under these conditions the organism remained alive for several hours, and ample opportunity was afforded for the study of its general morphology and activities. For the study of structural details, films were made on clean slides, allowed to dry, fixed in absolute alcohol and stained with Giemsa's solution; they were also stained with iron hematoxylin by Heidenhain's method, after fixation in hot Schaudinn's fluid.

GENERAL MORPHOLOGY

The organism is colorless and transparent, ovoid in shape, the anterior extremity being the broader. It is about twice as long as broad,

averaging approximately 20μ in length, although individuals vary considerably in size, the length ranging from 15 to 25μ. It has two cytostomes symmetrically situated on its lateral borders, extending from a point situated on the side and slightly on the ventral surface anterior to the middle of the body, to a short distance from the posterior extremity. The cytostomes are each limited by two thick lips, one of which is ventral, while the other is at the edge of the body. The anterior commissures of the cytostomes are sharp, and are located on the ventral surface, while the posterior commissures are round and more laterally situated. A small protuberance, from which the two cytostomic flagella arise, is sometimes visible at the anterior commissure. On each side, at a point situated slightly anterior to the origin of the cytostomic flagella, arises another flagellum, directed posteriorly, and to the side. This flagellum is of about the same length as the body of the organism.

A less conspicuous structure, resembling the axostyle of Trichomonas, is seen extending through the middle of the body. This is a colorless rod directed posteriorly from the region of the nucleus to the posterior pole of the flagellate. Its distal extremity is somewhat pointed, and sometimes protrudes from the posterior margin of the organism (Fig. 2), while at other times it appears to be drawn in (Fig.1). The posterior pole is usually smooth and rounded, but in some instances, round cytoplasmic processes are formed on each side of the posterior pole in the vicinity of the posterior cytostomic commissure (Fig. 5).

The cytoplasm is finely granular and contains numerous digestive vacuoles, in which bacteria and sometimes larger inclusions are easily detected. The vacuoles are usually seen in the anterior two-thirds of the body, although they sometimes appear in the posterior region. In fresh preparations stained with neutral red, the vacuoles were stained pale red, and could be observed in constant rotation throughout the anterior portion of the cytoplasm, but in no instance were food particles seen to enter or leave the organism. No contractile vacuole could be demonstrated. In other preparations, vitally stained with janus green, a dark line is visible near the anterior pole, following the curvature of the anterior border, and connecting the anterior commissures of both cytostomes. The nucleus could not be detected in fresh preparations.

In unstained fresh preparations, the organism moves rapidly across the microscopic field. Its behavior was most readily studied in preparations treated with janus green, which slows down the movements of the organism, without otherwise modifying them. It follows a zigzag course, going a short distance to the right, then to the left. When swimming free, or when attached to debris, it shows a constant rotatory motion around its longitudinal axis. It often became attached to a clump of bacteria by means of protoplasmic processes extruded from the posterior extremity. When this occurred, it would attempt to free itself by violent tugging movements.

The two anterior flagella, or "tractella" have a backward sweeping motion. After the death of the organism these flagella are directed posteriorly. Two short flagella of unequal length arise from each cytostome. One is of the same length as the cytostome, while the other is slightly longer. They exhibit an inward sweeping movement, the longer one reaching out further than the short one. It would appear that their purpose is to bring food particles into the cytostomic depression.

The nucleus has an average diameter of 4μ. It stains with difficulty with iron hematoxylin, and not at all with Giemsa's solution. In the best stained specimens, it appears to be of a granular type, and very poor in chromatin. It has a thin achromatic membrane with delicate granules scattered on its surface, and a few subcentral chromatic bodies of large size, but none sufficiently outstanding to be interpreted as a karyosome. A fine reticular network connects these granules with the nuclear membrane.

The flagella system is double and symmetrical. Each system arises from two definite blepharoplasts which stain readily with iron hematoxylin. The anterior blepharoplast is situated near the outer border of the body at a point about halfway between the anterior extremity and the middle of the body. A small compound blepharoplast formed by two minute closely related granules, is found at the anterior juncture of the cytostomic lips. The two blepharoplasts are connected by a thin filament which stains deeply with iron hematoxylin. The anterior, or large blepharoplast, gives rise to the long anterior flagellum, or "tractellum," which is directed outward and posteriorly. There is also a thick, heavily stained, curved rod, which, following the anterior cytoplasmic border of the organism, connects the two large anterior blepharoplasts. In stained preparations there is often a central break in the continuity of this rod indicating that it is made up of two similar component parts each of which may possibly be analogous with the basal rod described in other flagellates.

The posterior compound blepharoplast is located in the region of the anterior cytostomic commissure and is composed of two small granules. The two cytostomic flagella and a thick peristomal fiber arise from this blepharoplast. The cytostomic flagella are unequal in length and are directed posteriorly, lying close to the cytostome. The peristomal fiber outlines the contour of the ventral lip of the cytostome extending to the posterior commissure. In some stained preparations it has a granular appearance (Fig. 6).

The axostyle is an unstained structure that extends longitudinally through the middle of the body. Two small granules are regularly seen at the posterior pole, at the place where the axostyle protrudes from the cytoplasm (Figs. 3, 6, 7). Occasionally other chromatic granules are seen lying against the axostyle (Fig. 6).

CLASSIFICATION

This organism has apparently not been described as one of the intestinal parasites of the frog. Klebs (1893) described a free-living form 24 to 33μ in length, with two cytostomes and three flagella of unequal length on each side. Kleb's figures are from unstained preparations and although they represent an organism somewhat similar to the one described in this paper, I believe that I am dealing with a species different from the one named. Klebs named the flagellate *Trigonomonas compressa.* As no detailed description of the internal structures of *T. compressa* is given, it is very difficult to determine its relation to the flagellate here described. *T. compressa* is a free-living flagellate found in infusions, and showing a conspicuous contractile vacuole. The organism I found in the frog does not show a contractile vacuole, and seems pretty well adapted to parasitic life, although it cannot be definitely stated whether or not it is an obligatory parasite.

The name of *Trigonomonas diplostomum* is proposed for this form.

SUMMARY

A flagellated organism was found as an intestinal parasite in 30 per cent of the frogs examined. The flagellate was successfully cultivated on slants of N.N.N. agar base to which was added salt solution containing 0.5 per cent dried serum.

The organism was studied living after staining with vital stains and also in fixed films. Its general morphology and internal structure is described. It appears to be accidental parasite of the frog.

The name of *Trigonomonas diplostomum* is proposed for it.

Acknowledgment is made to Dr. Cesar Uribe for his suggestions in the study of this organism and assistance in the preparation of the plate.

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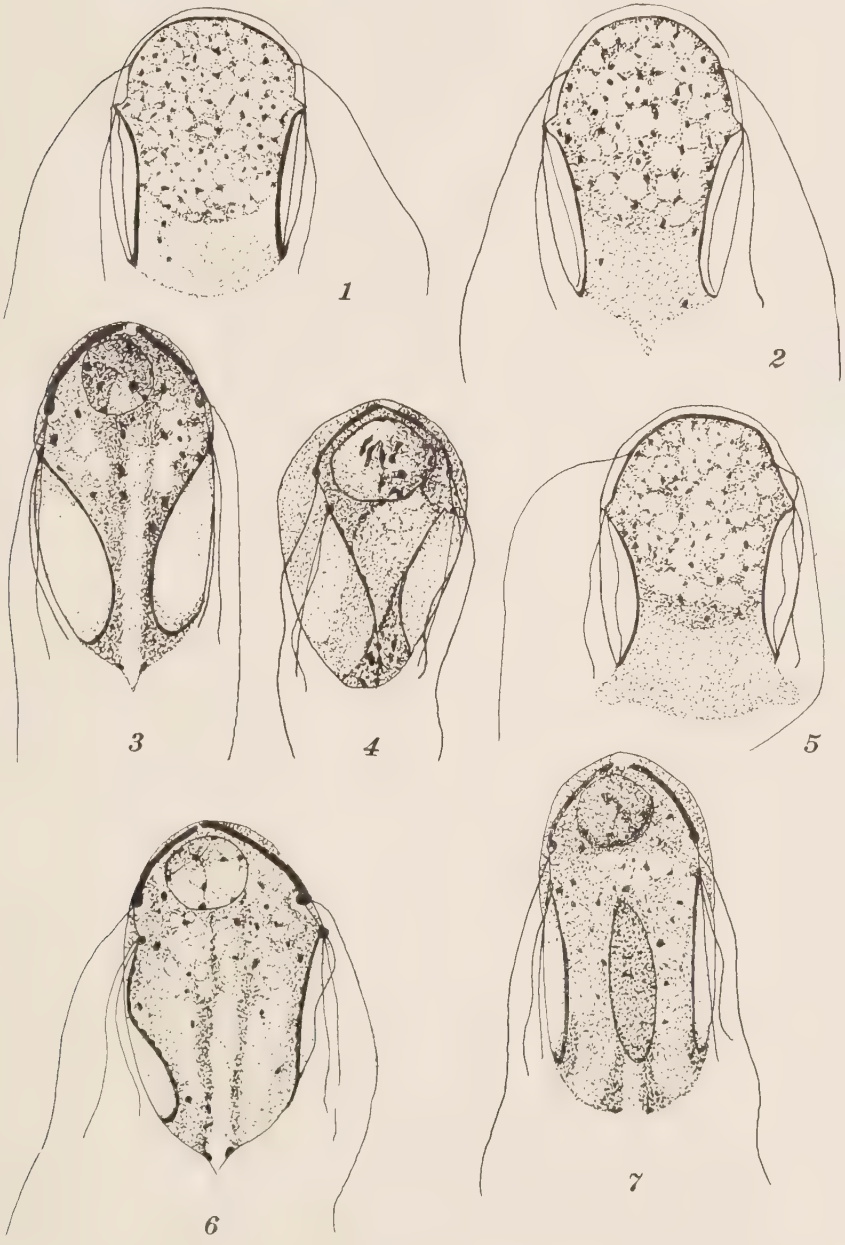

PLATE X

Figs. 1, 2, 5.—Preparations vitally stained with janus green.
Figs. 3, 4, 6, 7.—Stained with iron hematoxylin.

NOTES ON TWO SPECIES OF SOUTH AMERICAN TICKS, *ORNITHODOROS TALAJE* GUERIN-MENE., AND *ORNITHODOROS VENEZUELENSIS* BRUMPT

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The establishing of the new species of Argasine tick, *Ornithodoros venezuelensis,* by Brumpt, is, in my opinion, of considerable importance. This tick falls into the *Ornithodoros talaje* group and is very close to that species, but since Brumpt bases its separation on biological differences as well as on morphological characteristics, there seems to be no doubt in regard to the validity of *venezuelensis* as a species. However, it is now quite evident that in the past this species has been commonly mistaken for *talaje* and other closely allied species, especially by investigators of relapsing fever in South America.

The species of tick mentioned by Franco, Toro, and Martinez (1911) as *O. turicata* in connection with being the probable transmitting agent of relapsing fever at Muzo, Colombia, was very likely *venezuelensis.* This opinion is based on the illustrations accompanying their article as being more representative of this species than of *turicata,* and also owing to the fact that in 1924 I obtained a number of specimens of the Ornithodoros prevalent in Muzo, and they were all *venezuelensis.* No specimens of either *turicata* or *talaje* were present in the collection received from there.

The tick that Pino-Pou (1921) concluded to be the vector of relapsing fever in Venezuela and which he was inclined to consider as being *talaje* was also quite probably the species now known as *venezuelensis.* In 1921, while I was engaged in carrying out investigations on Ornithodoros as the intermediate host of relapsing fever in Panama, the ticks used in the experiments were classed as *talaje.* They followed so closely the description of *talaje* and compared so favorably with specimens in the collection at the Board of Health Laboratory at Ancon, C. Z., labeled *Ornithodoros talaje,* which for years had been believed to be typical of that species, that they were considered as coming under the same classification. After the investigations had been practically completed a few specimens of ticks, all nymphs, that differed slightly in appearance from those previously collected, were obtained. A comparison showed some minor differences, which, while not enough to satisfy me as being sufficient for basing a separation, nevertheless convinced me that, if they were not of different species, then the latter tick was the typical form of *talaje* and the one used in the relapsing fever work were a close variety.

Studies on the life histories of the two forms were then begun in order to ascertain if biological differences might be found which would prove them to be specifically distinct. However, owing to my departure from the Canal Zone soon after beginning these studies they were not carried out to an extent sufficient to be of much value in denoting any differences except on the period of attachment of the larvae. The larvae of the atypical form when placed on man or small animals became replete with blood and left the host in a comparatively short time, varying from twenty minutes to three hours, and showed no tendency toward remaining attached after becoming engorged.

The period of attachment of larvae of the typical form was observed only on wild rats. A number of brown rats, *Mus norvegicus,* captured in the city of Panama, that were heavily infested with larvae of the typical form were placed in large glass jars and watched closely to note when the ticks left the host. It was observed that some of the larvae did not detach themselves until as late as the fourth day after the rats had been placed in the cages. It was also quite probable that they had been attached for some time before the rats were captured. Molting occurred and the first-stage nymphs emerged in from two to six days after dropping from the rats; about 43 per cent molted on the third day. This would indicate either that the larvae require several days to become completely engorged or that a part of the molting period is passed on the host.

At the time of this investigation I was not aware that *venezuelensis* had been described and it was not until 1923, when after reading the description of this species for the first time, I found that it seemed to agree very closely with the tick which I had considered to be an atypical form of *talaje.* A comparison of the latter with specimens of *venezuelensis* which Dr. Brumpt kindly sent me showed no differences that I could discern. Specimens of the atypical form of Panama and also some that had been collected in Colombia sent to Dr. Brumpt for classification were placed by him as being *O. venezuelensis.*

Therefore, the ticks used in the transmission of relapsing fever in Panama as demonstrated by human experimentation by Bates, Dunn, and St. John (1921) and which were classed at that time (July, 1921) as *O. talaje,* should now be considered as *O. venezuelensis.* However, this should not be taken as indicating that *O. talaje* is not a transmitting agent of relapsing fever, but only signifies that the ticks used in the experiments as published in our report on the transmission as proven by human experimentation were *venezuelensis.*

Positive results were obtained in several inoculation experiments in which *talaje* nymphs that had been fed on rats infected with relapsing fever were allowed to molt and were then macerated and injected into fresh rats that later showed spirochetes in the blood stream. However,

the injection of macerated ticks, while of value in proving that they may be hosts of the spirochetes and capable of causing infection when injected or crushed on the skin, does not definitely determine them to be active transmitting agents. In view of this, some biting experiments were begun, but owing to lack of opportunity only two tests on the transmission by the bites of *talaje* (typical form) were carried out. One of these (No. 2) gave a negative and the other (No. 1) a positive result.

BITING EXPERIMENT NO. 1

Twenty-eight engorged larvae dropped from a gray rat, *Mus norvegicus,* No. 3, during June 24 and 25, 1921. Twenty-four of these molted and emerged as first-stage nymphs between June 26 and 30; the other four died before molting. A second molt occurred without a blood meal, twenty-two issuing as second-stage nymphs between July 2 and 10. Two more nymphs died during this second molt. On July 14, these

TABLE 1.—*Examination of Blood Films from Rat 386-2*

Date (1921)	Result	Number of Fields Examined
August 1*	Negative for spirochetes	600
August 2	Negative for spirochetes	600
August 3	Negative for spirochetes	600
August 4	Negative for spirochetes	600
August 5	Negative for spirochetes	600
August 6	Negative for spirochetes	600
August 7	Negative for spirochetes	600
August 8	Negative for spirochetes	600
August 9	Positive for spirochetes	Average, 1 in 181
August 10	Positive for spirochetes	Average, 1 in 88

* The examination on August 1 was made before the ticks had fed on the rat.

twenty-two second-stage nymphs were placed on white rat No. 17. This rat was infected with relapsing fever, having many spirochetes in its blood stream at this time. Twenty-one of the nymphs attached themselves and took blood, reaching various stages of engorgement. Two hours and six minutes was the longest time that any remained attached. After another molt, the third stage nymphs emerged between July 20 and 23. On August 1, they were placed on white rat No. 386-2. They all attached themselves and engorged in one hour and fifty minutes. Nearly all of them secreted coxal fluid freely.

Since the establishing of *O. venezuelensis* as a valid species, I consider (a) this tick to be the one commonly found in houses in various parts of Panama, Colombia, and Venezuela and possibly in some of the neighboring republics, and (b) that this species accepts man as its preferred host. It is also believed that *O. talaje* rarely attacks man and is seldom observed in dwellings, although probably present in considerable numbers. Numerous larvae, and occasionally a few first-stage nymphs of this species have been found on black and gray rats and it is my opinion that the nymphal and adult stages also accept rats, other

animals, and fowls as selected hosts, and man only as an adventitious host. This would account for the fact that no reports have been made of this tick being present and attacking man in some cities where the rats found in the houses were heavily infested with the immature stages. However, this is but an assumption on my part and is based more on circumstantial evidence than on conclusive observations.

In 1905, Darling (1922) found nymphs of *O. talaje* on black rats, *Mus rattus,* captured in buildings on Ancon Hospital grounds in the Panama Canal Zone.

Jennings (1912), in 1910, while making a series of observations upon the rats and their parasites in Panama, found many of the rats captured in the cities of Panama and Colon infested with numbers of the larval stage of *talaje.* He states, "from the abundance of the larvae upon Panama rats, the adults should be numerous in the houses of the poorer classes in that city, but efforts to secure specimens were unavailing."

In 1921, while carrying out an investigation on the prevalence and species of fleas on rats in the Canal Zone and Panama, I found many rats infested with *O. talaje.* Fifty-eight were taken from one brown rat, *Mus norvegicus,* captured at the Panama city dumping area. Twenty-one were collected from one taken in a tenement house on West 12th Street, Panama, and forty-seven were present on another captured at Dock No. 4, in Colon. These three instances serve to illustrate how heavy the infestation with ticks was in certain individual rats. Moreover, a high percentage of all rats examined were found to be infested to a certain extent. The brown rat seemed to be more heavily infested than the black. However, despite this large number of rats in the cities of Panama and Colon that were infested with larvae of *O. talaje,* I was unable to obtain specimens of the adult ticks in either of the cities.

The foregoing observations on the ectoparasites of the rats in Panama show that the larval and nymphal forms of *O. talaje* have been infesting the rats on the Isthmus for more than two decades. Notwithstanding this fact, the adults apparently have not been commonly found in the houses in the cities of Panama and Colon and attacks on man by this species were not reported in these cities. In August, 1921, I secured a few adults of this species from the native villages of Chorrera and San Juan, in the Republic of Panama. A predominating number of *O. venezuelensis* were also present in the collections from each of these villages. These specimens were collected and sent to me by a native and, unfortunately, no detailed information regarding the places of collection was obtained.

In 1924, while investigating relapsing fever in Colombia, I secured a total of 4,880 specimens of *O. venezuelensis.* This number represented

collections from sixty-eight houses in twenty villages, towns and cities in various parts of the republic. During the course of these collections *O. talaje* was only encountered in three houses. Two of these were of the cane wall and thatched roof type of houses and were located in Barranquilla. Fourteen *O. talaja* and twenty-one *O. venezuelensis* were found in one, and ten *O. talaje* in the other. These specimens from Barranquilla were collected by a young man living in that city and who, after receiving some training in the work, became quite expert in locating the ticks in houses where only a very few were present. The third house in which *O. talaje* were found was located at Soledad, a small town a short distance from Barranquilla. The gentleman occupying this house brought me three specimens. These had been obtained only after a considerable search and were found in crevices in the upper part of the walls near the roof of the kitchen.

The location of these hiding places seems to vary somewhat from that of *O. venezuelensis,* as the latter species were usually found in the living or sleeping rooms. Furthermore, when present in the walls I have never found them up near the roof. It is possible that in this instance the thatched roof of the kitchen was frequented by rats and that they had been the source of blood supply for the ticks. The collector informed me that neither he nor his family was aware of ever having been bitten by these ticks. No *O. venezuelensis* were found in this house.

I examined but one gray rat, *Mus norvegicus,* in Barranquilla and found that to be infested with four larvae of *O. talaje.* This rat had been captured near the business district of the city. It is quite probable that if there had been an opportunity of examining a number of rats at Barranquilla they would have been found to be as heavily infested with the larval form of this tick as are the rats in Panama.

SUMMARY

Two species of *Ornithodoros, O. venezuelensis* and *O. talaje,* are prevalent in various parts of northern South America. Biting experiments have shown that both these ticks may transmit relapsing fever. It is believed that *O. venezuelensis* is the principal transmitting agent of this disease in man in tropical America.

Since *O. venezuelensis* are found in considerable numbers in many human habitations, where they attack the occupants, it is considered that man is the selected host of this species.

The larvae of *O. talaje* are commonly found on rats in some parts of South America and it is assumed that the nymphs and adults usually attack rodents and larger animals and that man is accepted only as a host of necessity.

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HOST-PARASITE SPECIFICITY IN THE COCCIDIA OF MAMMALS*

JUSTIN M. ANDREWS

The problem of host-parasite specificity in the coccidia undoubtedly ranks next to that of therapeutics from an economic and medical standpoint. Man is concerned to know whether or not he may be infected with the coccidioses which are so abundant among his domestic animals or household pests. For the sake of his domestic animals, he inquires whether or not the disease which is so devastating among his cattle is identical with the infection among the rats and rabbits, and also whether the flying birds such as sparrows carry a coccidial organism which is infective to poultry. In other words, it is important to know whether or not certain animals can act as biological or mechanical vectors of coccidiosis in man or his domesticated animals. The importance of this question is even greater from a purely scientific point of view. Granted that there is such a phenomenon as specificity between host and parasite, to what extent does it prevail and by what mechanism does it operate?

Many assertions have been made regarding host-parasite specificity in the coccidia, especially those that infect man, and some experimental work has been done. The assertions have been based on morphological resemblances, and on the fact that certain parasites occupy similar tissues in different hosts.

Until recent years, parasitologists have generally believed that the coccidia of man, rabbit, cat and dog belonged to the same species, and that man contracted his coccidial infections from the lower animals. Dobell (1919: 193) who has painstakingly analyzed all of these previous accounts, concluded with the following statement: "All of these coccidia [referring to those described from man] are probably peculiar to man. There is no evidence that any of them is or can be parasitic in any other host."

There is little experimental information concerning host-parasite specificity, and some of that is probably not to be trusted. Cross-infectivity seems to be an easily testable experiment to make, and yet the results may be very misleading unless one bears in mind three things: first, the natural coccidial parasites of the animal, second, the interference of an acquired immunity, and third, mechanical carriage. The first difficulty amounts to very little if the foreign parasites employed differ decidedly in their morphology from the coccidial para-

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sites which are natural to the experimental host. In certain cases, however, oocysts, especially in the unsegmented condition, of species of coccidia from different hosts (e. g., the three species of Isospora from cats and dogs), are so similar that it would be unwise to rely upon the visual test to distinguish them.

The interference of immunity by virtue of a previous infection is a problem the significance of which is hard to estimate at present. It has been shown (Andrews, 1926) that it is not possible to reinfect a cat or dog with a species of coccidium by which it has been previously infected. Just how long this immunity lasts is uncertain, but the evidence indicates that it remains for several months and probably for years. Furthermore, it is not yet known whether infection by one species confers immunity from infection by other related species or genera as well. It seems improbable, however, that this latter is the case to any marked extent judging from other instances of protozoan immunity (trypanosomiasis). Furthermore, coccidial infections of two or more species may occur simultaneously in the same animal (cat, dog, rabbit) without appearing to interfere with each other, as far as may be judged by the course of the infections. It is obvious that this interference will be of greater importance in dealing with species of coccidia found in hosts of closely related or intimately associated species. It would, for instance, be of considerable significance in determining the host specificity of the coccidia of the cat and dog, but would probably be of little importance when attempting cross-infections with the coccidia of the opossum and rabbit.

The third point is well illustrated in the recent contributions of Thomson and Robertson (1926a, b) who state that the appearance of Eimeria oocysts in the stools of humans is due to the ingestion of small fish (sprat, mackerel, herring, sardines) the testes and livers of which have been shown to contain enormous numbers of oocysts that are morphologically identical with the oocysts of the Eimeriae described from man (Dobell, 1919, 1921).

The establishment of positive cross-infectivity in all these cases requires, therefore, the successful inoculation of young animals (with coccidia from a foreign host) that are free and have been free from coccidiosis from birth, and the careful examination, day by day, of the feces of these animals so that if an infection occurs, its course can be followed and the prepatent and patent periods * compared with what would be expected in an infection of the animal by its natural parasites.

* The *prepatent* period in coccidiosis is defined as the time from the entrance of the inoculating oocysts until the appearance of new oocysts in the feces as determined by a stated method.

The *patent* period is the time from the appearance to the disappearance of discharged oocysts as determined by a stated method. See Hegner (1926) and Andrews (1926).

The following table (Table 1) summarizes the available literature on the cross-infectivity of coccidia from various hosts.

From these data it may be inferred that the coccidia of mammals are strictly host-specific parasites. Moreover, the one exception noted (Fantham, 1917) is probably of little significance. No details of the infection of the kitten by the oocysts from man are given and it is, therefore, possible that the natural coccidial parasites of the kitten were not excluded. Furthermore, Wenyon and O'Connor (1917) duplicating the experiment the same year arrived at just the opposite conclusion and mentioned in their report the occurrence of coccidia in kittens, the oocysts of which they distinguished from those of *Isospora hominis*.

In considering these data on cross-infectivity, some possible error may have occurred since scarcely any of the workers were aware of the

TABLE 1.—*Reports of Cross-Infectivity Experiments with Coccidia of Mammals*

Observer	Date	Natural Host	Experimental Host
a. Successful attempts			
Fantham	1917	Man	Kitten
b. Unsuccessful attempts			
Wenyon and O'Connor	1917	Man	Mouse
Wenyon and O'Connor	1917	Man	Kitten
Galli-Valerio	1918	Cattle	Rabbit
Hall and Wigdor	1918	Dog	Rat
Bruce	1920	Rabbit	Cattle
Bruce	1920	Cattle	Horse
Bruce	1920	Cattle	Pig
Bruce	1920	Cattle	Sheep
Bruce	1920	Cattle	Rabbit
Bruce	1920	Cattle	Guinea-pig
Bruce	1920	Cattle	White rat
Noe	1920	Man	White rat
Böhm	1923	Dog	Man
Böhm	1923	Dog	Kitten
Böhm	1923	Cat	Rat
Böhm	1923	Cat	Dog
Nöller (quoted by Böhm, 1923)	....	Cat	Dog

immunity which is acquired by infection. Indeed, it has been the accepted view that coccidiosis does not confer any appreciable immunity, and that what appear to be chronic sustained infections are actually a continuous series of reinfections (Wetzel, 1925). While this concept has been applied to the coccidioses of mammals in general, it appears to be derived principally from a study of rabbit coccidiosis, where this situation undoubtedly prevails (Reichenow, 1921). Its general applications hardly seem to be justified, at least in the case of cats and dogs (Andrews 1926). It is, therefore, possible that some of the experimental animals were not susceptible to their own coccidial parasites, and that this immunity may have likewise protected them from infection by some of the closely related species of foreign coccidia.

The following observations tend to support, in general, the results to which reference has just been made. They fall into three groups, (1) the interchangeability of Isosporae of cats and dogs, (2) cross-infec-

tivity of coccidia of various mammals, and (3) selective digestion as a factor in the mechanism of host-parasite specificity.

1. The interchangeability of Isosporae of cats and dogs

On the basis of morphology, the three species of Isospora, *I. felis* Wenyon, 1923; *I. rivolta* (Grassi, 1879), and *I. bigemina* (Stiles, 1892) which occur in cats seem to be the same three species which occur in dogs. The oocysts of a given species from the three different hosts are similar in form and size range. If they are identical species, it follows that they should be infective to both hosts. During the early part of this work, it was assumed that this was true, and it was not until very recently that some doubt seemed to be cast upon this assumption by the experiments of Böhm (1923). He fed some of the same batch of oocysts, that had proved to be infective to a mongrel dog, to a kitten and to a man. Though the feces of both these experimental animals were examined for a month, no oocysts were found. To one of two two-months old fox-terrier puppies, he fed oocysts (*Isospora felis*) on three consecutive days. The other dog was kept for a control animal. The feces of both animals were examined for sixty-seven days after the first feeding, but oocysts were never found in either. These seem to be the most conclusive experiments on record concerning the interchangeability of the coccidial parasites of cats and dogs, and strongly suggest that there are two different kinds of parasites, the one of which is infective to cats but not to dogs, and vice versa.

The following extracts from protocols indicate my own work on this point. Some of them happen to contribute information on this subject in a purely incidental way, although the last seven notations represent deliberate attempts to investigate the interspecificity of cat and dog coccidia.

Dog 19. Acquired 7/11/25—mongrel puppy, 6 weeks old. Feces negative for over two weeks. (This animal was one of my first, and my technique for isolating and handling animals was undeveloped and inefficient.)

Diarrhea appeared 7/20 and lasted four or five days. Oocysts of *I. felis* and *I. rivolta* were found in feces 7/28 and 7/30, but not thereafter.

8/5—given 100,000 oocysts of *I. felis* and *I rivolta.* Feces remained negative.

8/28—given 10,000 oocysts of *I. felis* and *I. rivolta.* Feces remained negative.

9/30—given intraduodenal injection of living merozoites of *I. felis from* Cat No. 56. Feces negative thereafter.

All fecal examinations by smear method.

This animal must have acquired its infection from cats as it was the only dog I had at the time.

Dog 49. Acquired 8/7/25—mongrel terrier puppy, 2 months old. Feces negative for over a week.

8/17—passed oocysts of *I. rivolta,* and five days later *I. felis* as well. Contined to discharge oocysts of both species until 9/4. Feces negative thereafter.

9/21—given 20,000 oocysts of *I. felis* and *I. rivolta.* Feces remained negative.

10/21—given 10,000 oocysts of *I. felis* and *I. rivolta* from cat. No signs of coccidiosis clinically or parasitologically thereafter.

All fecal examinations by smear method.

This animal probably acquired its infection from cat feces, as it was first put into a cage which may not have been thoroughly cleaned. The only other dog I had at that time was No. 19 (see above) and it had apparently ceased to discharge oocysts when No. 49 was acquired. The dogs were not permitted to be together until No. 49 had recovered from its infection.

Dog 90. Acquired 10/12/25—Irish terrier, about 10 weeks old. Feces negative for over three weeks.

11/6—inoculated with *I. rivolta* from cat. No oocysts or symptoms thereafter.

All fecal examinations by smear method.

Dog 99. Acquired 10/23/25—Irish terrier, 6 weeks old. Feces negative for over ten days.

11/6—inoculated with fecal suspension from Cat. 104, containing ripe oocysts of *I. felis, I. rivolta,* and *I bigemina.* Diarrhea third and fourth days after inoculation, but no oocysts were ever found in feces.

All fecal examinations by smear method.

Dog 107. Acquired 11/3/25—fox terrier, 3 months old. Feces negative.

11/4—inoculated with *I. rivolta* from cat. Diarrhea from 11/7 to 11/11. Feces examined for twenty-eight days after inoculation but no oocysts were ever found.

All fecal examinations by smear method.

Dog 108. Acquired 11/3/25—fox terrier, 3 months old. Feces negative.

11/4—inoculated with 200,000 oocysts of *I. felis* and *I. rivolta* from cat. Diarrhea 11/9. Feces examined for twenty-two days after inoculation, but no oocysts were ever found.

All fecal examinations by smear method.

Dog 126. Acquired 1/8/26—mongrel puppy, about 5 months old, raised in laboratory. Feces negative. Inoculated with 20,000 oocysts of *I. felis* and *I. rivolta* from cat. Oocysts of *I felis* and *I. rivolta* discharged from 1/18 to 1/31. Feces negative thereafter.

2/26—inoculated with *I. felis* from dog. No clinical or parasitological evidence of *I. felis* for at least fourteen days thereafter.

All fecal examinations by D. C. F.* method. Oocysts were very few, and it is very improbable that they would have been discovered by the smear method.

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* The direct centrifugal flotation method (D. C. F. in protocols; a modification of Lane's technique for the detection of hookworm ova) for the detection of coccidial oocysts is as follows. Strong Erlenmeyer flasks of sixty-five or seventy cubic centimeters capacity were calibrated and etched to indicate volumes of fifty-six and fifty-eight cubic centimeters. The flask is filled with water up to the level of the fifty-six cubic centimeter mark, and then a sufficient amount of the specimen stool collected at random from various parts of the feces is added to bring the fluid level up to the fifty-eight cubic centimeter mark. Thus two cubic centiemters of the stool have been added. Four or five cubic centimeters of lead shot (medium size and small, mixed) are poured into the mixture. The flask is closed with a rubber stopper and shaken until the contents are uniformly mixed. If the stool is hard, it is futile to try to comminute it by shaking with shot unless it has stood in water for several hours. The most convenient routine found was to let the stoppered flasks stand with their contents overnight, and to shake them the following morning. The thoroughly emulsified specimen is then poured into a round-bottomed fifty cubic centimeter centrifuge tube to a mark which has been etched to indicate a volume of forty-five cubic centimeters. The centrifuge tubes are then balanced in their cups by the *addition* of water, and are whirled at the lowest speed of the centrifuge (International Electric Centrifuge, Size 1) for two or three minutes. This manipulation sends all of the oocysts to the bottom of the

(Footnote continued on following page)

Dog 145. Acquired 2/21/26—Irish terrier, 2 months old. Feces negative for several days.

2/26—Inoculated with *I. felis* and *I. rivolta* from cat. No oocysts detected in feces until 3/7. Diarrhea from 3/2 to 3/7. A very few oocysts of *I. felis* and *I rivolta* were passed sporadically until after 3/18. Feces negative thereafter.

3/31—inoculated with *I felis* from dog. No oocysts of *I. felis* have been seen in feces since inoculation.

All fecal examinations by D. C. F. method.

Cat 152. Acquired 3/5—four weeks old. Feces negative. Inoculated with *I. felis* and *I rivolta* from dog. No oocysts found until 3/22. Continues to pass oocysts of *I. felis* and *I. rivolta* and has intermittent diarrhea.

All fecal examinations by D. C. F. method.

These data are summarized in Table 2, in which diarrhea is taken as the clinical manifestation of coccidiosis. This sign is only a presumptive indication, but the finding of oocysts in the feces day after day is indisputable parasitological evidence of coccidiosis. From this table,

TABLE 2.—*Cross-Infectivity of Cat and Dog Coccidia*

Dogs inoculated with coccidia from cats Cats inoculated with coccidia from dogs		
Animal	Clinically	Parasitologically
a. Fecal examinations by smear method		
Dog 19	Positive	Positive
Dog 49	Negative	Positive
Dog 90	Negative	Negative
Dog 99	Positive	Negative
Dog 107	Positive	Negative
Dog 108	Positive	Negative
b. Fecal examinations by D. C. F. method		
Dog 126	Negative	Positive
Dog 145	Positive	Positive
Cat 152	Positive	Positive

it appears that, of eight dogs inoculated with coccidia of feline origin, four gave parasitological evidence of coccidiosis, and, of the four remaining, three gave a clinical sign that might have been confirmed parasitologically had the D. C. F. method of diagnosis been employed at the time. The identity of the species of coccidia in the cat and dog is also indicated by the fact that dogs 126 and 145, having recovered from coccidiosis of cat origin, were refractory to infection by oocysts of the same species from dogs. Unfortunately I have had only one kitten available to test with dog coccidia. That case, however, is very clearcut and lends confirmation to the argument that the coccidia of cats and

tube. The supernatant fluid is carefully decanted, all of the sediment being retained. A boiled saturated solution of sodium chloride is then added in small amounts while the tube is gently shaken so that the sediment is loosened and mixed with saline. About forty-five cubic centimeters of the solution are added in all. The tubes are then balanced by the addition of the saturated saline and centrifuged at the same rate of speed as before, but no longer than minute. A portion of the upper layer of the mixture is lifted off by suspension across the mouth of a small vial on to a glass slide, and is quickly examined under the microscope. If oocysts are present, they will be found floating in the surface layer.

dogs are probably of the same species, and are mutually interchangeable. The uniform mildness of the infections with the cat oocysts in the dogs, with the exception of Dog 49, suggests an aberrant type of infection due to the unnatural host. This may also account for the unusually long prepatent period in Cat 152.

2. *Cross-infectivity of coccidia of various mammals*

In the following experiments, the interference of an acquired immunity has been reduced to the least practical minimum by exercising the utmost care in the selection of experimental animals. Inasmuch as there was no indication of positive cross-infections, it is clear that there

TABLE 3.—*Attempts at Cross-Infection*

Experimental Animal No.	No. of Trials	Period of Examination	Kind of Oocysts	Source of Oocysts
Dog 19	7	92 days after 1st inoculation	Eimeria perforans and E. stiedae	Rabbit
Cat 32	4	92 days after 1st inoculation	E. perforans and E. stiedae	Rabbit
Dog 58	1	10 days	E. perforans and E. stiedae	Rabbit
Dog 111	1	56 days	E. perforans and E. stiedae	Rabbit
Dog 112	1	39 days	E. perforans	Rabbit
Dog 113	1	45 days	E. perforans and E. stiedae	Rabbit
Skunk 118	1	23 days	E. perforans	Rabbit
Skunk 119	1	23 days	E. perforans	Rabbit
Cat 120	1	19 days	E. perforans and E. stiedae	Rabbit
Cat 121	1	13 days	E. perforans and E. stiedae	Rabbit
Cat 122	1	19 days	E. perforans and E. stiedae	Rabbit
Cat 123	1	19 days	E. perforans and E. stiedae	Rabbit
Cat 125	1	14 days	E. perforans and E. stiedae	Rabbit
Opossum 127	1	7 days	E. perforans	Rabbit
Opossum 128	1	22 days	E. perforans	Rabbit
Opossum 129	1	8 days	E. perforans	Rabbit
Opossum 130	1	12 days	E. perforans	Rabbit
Dog 138	1	12 days	E. sp ?	Skunk
Cat 150	1	28 days	E. sp ?	Prairie-dog
Cat 156	1	10 days	E. debliecki	Pig
Cat 157	1	12 days	E. sp ?	Skunk

are no misinterpretations on the basis of natural coccidial parasites or because of mechanical carriage.

The data regarding attempts at cross-infection are presented in Table 3. The cats, with the exception of the first (32) were all raised in the laboratory, and it is positively known that Cat 32 had not had coccidiosis before it came to the laboratory because it developed an infection with *I. felis* subsequent to its inoculation with eimerian oocysts from the rabbit. All of the dogs used in the experiments were acquired by the time they were ten weeks old. With the exceptions of dogs 58 and 138, they had all been infected with Isospora in the laboratory before cross-infections with Eimeria (from another host) were attempted. None of them, however, had ever shown any signs of natural infections with Eimeria. It is not known whether the immunity which they had acquired by virtue of their isosporan infections, and which would certainly have rendered them refractory to further infections by the same species, would protect them against infection by Eimeriae from foreign

hosts. Attempts to infect Dog 58 with Eimeria from the rabbit preceded infection with natural isosporan parasites. Of the history of Dog 138 with respect to coccidiosis previous to his laboratory experiences, nothing is known, and it was not possible to test his susceptibility to dog coccidia after attempting an infection with the Eimeria of skunks as he was stolen from his cage twelve days after his inoculation. The opossums were all half-grown animals. Nothing is known of their previous history with regard to coccidiosis. The two skunks were adult animals, and each had a very light infection of *Eimeria* sp., the oocysts of which were decidedly different in appearance (spherical or subspherical) from the foreign parasites, *E. perforans* from the rabbit (elongate oval) which were introduced. Whether or not this chronic infection was responsible for the failure to successfully cross-infect these animals is not known.

The feces of all these animals were carefully examined for periods varying from a week up to three months. Oocysts of the foreign coccidia were frequently seen during the first fortiy-eight hours after inoculation, but these were not regarded as being significant and so are not included in the table. The fecal examinations for the first three animals (Dogs 19 and 58 and Cat 32) were made by the smear method: all of the rest were by the D. C. F. method. After death, the intestines of these animals were carefully examined for evidences of coccidiosis, but in every case the fecal diagnoses have been confirmed. In none of these cases was there any indication that the oocysts of one kind of mammal were able to successfully infect another kind of mammal whether it had ever had coccidiosis before or not. The only exception to this generalization seems to be in the case of cats and dogs, whose coccidal parasites seem to be reciprocally infective. These results are in harmony with and materially substantiate the results of other workers quoted in Table 1.

3. Selective digestion as a factor in the mechanism of host-parasite specificity

The host-parasite specificity of the coccidia of mammals seems to be such an exact phenomenon that some work has been done in attempting to get at the nature of the mechanism by which this remarkably precise selection operates. It is recognized at the outset that host-parasite specificity is the result of reactions on the part of the host and to no less degree on the part of the parasite, and it is probably no one set of reactions on the part of both, but a combination of several. Infection by coccidia involves two separate processes, excystation and tissue penetration so that one might reasonably look for the protective mechanism either in the digestive processes, or in the formed or unformed elements of the blood or invaded tissue. It is the purpose of this section to consider only the first group of possibilities, namely, selective digestion.

It is conceivable that such a process might operate in either one of two ways. The digestive fluids of the foreign host might be unable to affect the oocyst wall so as to release the contents during the time that the organisms are present within the intestine. On the other hand, the digestive ferments of the foreign host might be so vigorous that they not only released the sporozoites promptly, but attacked them so effectively that they either destroyed them or, what is quite as unfortunate from the point of view of the parasite, paralyzed them so that they are unable to penetrate tissue.

Some strength is lent to these speculations by the fact that the intestinal coccidia of mammals are quite clearly divided generically by the eating habits of the host. Thus herbivorous mammals are exclusively parasitized by species of Eimeria, while the flesh-eating mammals are usually parasitized by Isospora. So far I have seen no references to the occurrence of Isospora in herbivorous mammals, though there are one or two exceptions to the converse of the rule. The Eimeria which I have found in two skunks is undoubtedly a true parasite of these carnivores, and it may be that the exceedingly doubtful species, *E. felina* Nieschulz 1924, and *E. canis* Wenyon 1923, from the cat and dog will have to be included. In general, however, Eimeria parasitizes a digestive tract specialized for the manipulation of vegetable foods, and Isospora occurs in intestines designed to handle fleshy foods. It is clear then that the oocysts of each genus ordinarily excyst and infect under two exceedingly different types of intestinal environment. Furthermore, the finding of many rabbit oocysts in the feces of cats and dogs strongly suggested that the oocysts were unaffected by the intestinal fluids of the foreign hosts, at least during the length of time that they were in the intestine. These observations seemed to indicate that such a phenomenon as selective digestion might play a part in the mechanism of host-parasite specificity. To test this hypothesis, a series of experiments were performed in which were suspended, in various sorts of containers in the stomach or duodenum of an animal both native and foreign parasites. The oocysts were subsequently recovered to determine the effect of the digestive juices on each kind after various intervals.

The cats and dogs were anaesthetized either with ether or with morphine and novocaine, laparotomized, and a gastrotomy or duodenotomy performed. The containers were anchored by means of a cotton suture passed through the mucosa. If reasonable antiseptic conditions were maintained, there was no difficulty about recovery. Occasionally it was necessary to irrigate the wound for a few days after the operation with warm Dakin's solution. Reentries for the recovery of the parasites were made through the original abdominal incision. Various sorts of containers such as collodion sacks, short pieces of glass tubing plugged with cotton, and bags made of finely woven cotton cloth were experimented

with, the most satisfactory of which was made of two layers of nainsook separated by a piece of thin filter paper. A wisp of cotton saturated in the liquid containing the oocysts always retained enough of the organisms for subsequent examination.

After exposure to the digestive fluids for a certain length of time, the occysts showed certain changes which have been interpreted as the effects of digestion. The enveloping wall of the organism (Isospora) appeared to have become softened and collapsed against the sporocysts. The sporocyst wall showed similar signs of swelling and softening. Frequently the oocyst was ruptured, and one or both sporocysts were missing. The changes in the wall of the oocysts of Eimeria were observed, though they were not so marked, but empty oocysts were frequently found after sufficient exposure to digestion. Observations on the conditions of the oocysts were made with two series of animals

TABLE 4.—*Selective Digestion of Natural and Foreign Coccidial Parasites of the Cat and Dog*

Animal No.	Exposure to Digestion	Eimeria (Foreign)	Isospora (Natural)
Series 1.			
Cat 54	11 hours	Normal	Normal
Cat 52	23 hours	Normal	Slightly digested
Cat 18	48 hours	Normal	Digested
Cat 41	72 hours	Slightly digested	Digested
Series 2.			
Dog 68	12 hours	Normal	Slightly digested
Dog 66	24 hours	Slightly digested	Digested
Dog 63	48 hours	Digested	Digested
Dog 64	72 hours	Digested	Digested

N. B. Normal, none of the oocysts affected; slightly digested, a few show evidences of digestion, but by far the majority of them are unaffected; digested, by far the majority of the oocysts show definite evidences of digestion.

at intervals up to seventy-two hours. The results are expressed in Table 4.

It will be noted that, while the results in the two series are quite comparable; excystation of both kinds of parasites takes place in the dog about twenty-four hours sooner than in the cat, due, perhaps, to the more vigorous action of the digestive fluids of the dog. The most interesting point is that in both series of animals the natural parasites showed the effect of the digestive action in from twelve to forty-eight hours before the foreign parasites showed similar evidences of excystation. Taking into consideration the length of time that material must remain in the intestine, it seems probable that this margin in the differences of reaction of the natural and foreign oocysts to the digestive fluids may be of some value in protecting the animal from infection by foreign coccidia.

These results having been obtained with digestion *in vivo*, the following experiment was designed to find out whether they could be duplicated *in vitro*. After stimulating gastric secretion by the administration

of milk, the stomachs of dogs 66 and 68 were pumped out. One cubic centimeter of a suspension of Eimeria oocysts, and a similar one of Isospora, was diluted with two cubic centimeters of the filtered gastric juice which had been aspirated. These tubes were placed in an incubator at 37.5 C, a duplicate set being left at room temperature. These were observed at various intervals up to three days. No change in the appearance of the oocysts, either foreign or natural, could be made out in the contents of any of the tubes.

If selective digestion does play a part in protecting an infectible animal against promiscuous coccidial parasites, it is probably not the entire mechanism, as the following experiments (Table 5) suggest. It is clear that, if the protection of an animal lies only in the greater resistance of the foreign oocysts to the digestive processes of the animal, it should be easy to infect a foreign host by inoculating it with merozoites, which, presumably, do not require a digestive process to render them

TABLE 5.—*Inoculation of Cats and Dogs with Foreign Merozoites*

Animal No.	Species of Merozoite Injected	Source	Period of Examination
Injected intraduodenally			
Dog 58	E. perforans	Rabbit	7 days
Cat 62	E. perforans	Rabbit	14 days
Dog 111	E. perforans	Rabbit	40 days
Dog 112	E. perforans	Rabbit	28 days
Cat 134	E. perforans	Rabbit	30 days
Cat 163 (control)	I. felis	Cat	Positive after 5 days
Injected intrahepatically			
Cat 133	E. stiedae	Rabbit	33 days

infective. The method of doing this was to laparotomize a cat or a dog, and to inject into the duodenum the scrapings from the intestinal mucosa of a rabbit infected with *E. perforans*. In one case, comminuted liver substance containing the merozoites of *E. stiedae* were injected into the liver of a cat.

Inasmuch as uninfected rabbits were not available, the only guarantee of the viability of the merozoites, which were suspended in warm saline, was the fact, that they were motile up to the time they were injected. Thus while it was not possible to control the experiment by inoculating an uninfected rabbit with merozoites from another rabbit, the possibility of the asexual transfer of coccidiosis was demonstrated by inoculating a kitten, known to have been previously uninfected, with the merozoites of *I. felis*. The animal showed a typical infection.

None of these animals ever showed any indication of an Eimeria infection, which indicates that if the selective digestion of oocysts does play any part in the mechanism of host-parasite specificity, it is supplemented by some other process. It is possible, as indicated above, that selective digestion may operate by disposing of foreign merozoites.

CONCLUSIONS

1. *Isospora felis* and *I. rivolta* from the cat and dog appear to be infective to both animals, though the course of the infection varies in each.

2. With the exception noted above (1), the coccidia of mammals seem to be strictly host specific parasites, as judged by cross-infectivity experiments on cats, dogs, rabbits, skunks, opossums, pigs, and prairie-dogs.

3. It has been shown that excystation of the oocyst is facilitated by the digestive processes of the natural host, but that in the foreign host oocysts are so resistant to digestive action that the sporozoites are not released during the normal length of time that the organisms pass through the intestine.

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A NOTE ON CHARCOAL CULTURES FOR HOOKWORM LARVAE *

L. H. Vidhikar

It is generally accepted, following Looss (1911), that powdered animal charcoal or bone black mixed with human feces make the best cultures for infective hookworm larvae. The author, using standardized 3 gram cultures of the type described by Stoll (1923), which involve also preliminary dilution egg counts and isolation of the larvae with the Baermann apparatus, has secured results on 318 cultured bearing on the choice of a charcoal for culture, and the proportions of the charcoal-feces mixture. These data permit a rather clear-cut decision in favor of blood charcoal rather than bone black, but indicate as well that for neutral stools, derived from an individual living on a mixed diet, more larvae are obtained without any charcoal. It is a pleasure to convey my thanks to Dr. W. W. Cort and Dr. Norman R. Stoll for their general direction of and interest in this work.

MATERIALS AND METHODS

The stools used were all obtained from one person, Case A, which is held to be a control factor of importance. This individual was a well-nourished adult living on a mixed diet, with a mixed infection of both *Ancylostoma duodenale* and *Necator americanus*. The specimens were transported to the laboratory in the morning, kept at room temperature, and cultures made in the early afternoon of the same day. The feces were usually soft formed in consistency, yellow or sometimes brownish in color, often contained some undigested vegetable debris, and varied in amount per specimen from 60 to 250 grams. So far as tested these specimens were practically neutral from the standpoint of acidity. The stools before being used for culture and egg count were thoroughly stirred on a large glass slab. Egg counts averaged between 1,000 and 2,400 eggs per gram. In all 26 stools were cultured.

The charcoals employed were as follows:

Blood charcoal. Source: J. T. Baker Chemical Co. It had an acid reaction with p_H reading † of 6.1, was lighter in weight than the animal charcoal and absorbed water more easily.

Bone black or powdered animal charcoal. Sources: Eimer and Amend, and A. H. Thomas and Co. A fine black powder which had an alkaline reaction of p_H 7.6.

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* Contribution from the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

† I am indebted to Dr. Olaf S. Rask for assistance in making the colorimeter tests for the p_H of the charcoals.

Animal charcoal, washed with acid. Source: J. T. Baker Chemical Co. A gray coarse powder that was distinctly acid at p_H 5.6.

Granular animal charcoal. Source: A. H. Thomas Co. This was the coarsest charcoal used and seemed to be a combination of lustrous black and white crystals. It required more water than the others in order to bring it to the consistency of butter, and had a p_H of 6.9.

Willow wood charcoal. Source: Muth Brothers and Co. of Baltimore. This was a fine black powder, absorbed water very slowly, and was alkaline, with p_H 7.5.

General culture and isolation methods have been given by Stoll (1923) and need not be repeated here. The cultures were kept in an incubator which was maintained at 29° to 30°C. At first cultures were isolated after 4, 5, 6 and 7 days. The four day cultures with all charcoals were consistently lower than those from the other three days, but as between the latter no valid differences were noted. Consequently four day cultures are omitted from the analysis and those of 5, 6 and 7 days used as equivalent.

For biometric methods the reader is referred to Pearl (1923).

TABLE 1.—*Comparison of Various Charcoals Used in 3 Gram Hookworm Cultures*

Cultures with	No. of Cultures	Larval Percentages of Original Egg Counts: Poorest Culture	Best Culture	Mean ± P. E.	Coefficient of Variation All Cultures
Feces only (controls)	50	13	78	40 6 ± 1.7	42.7 ± 3.4
Blood charcoal	38	8	73	32.4 ± 1.5	43.5 ± 4.0
Bone black (powdered animal charcoal)	41	6	46	24.8 ± 1.1	42.3 ± 3.7
Granular animal charcoal	6	14	27	17.8 ± 1.3	26.1 ± 5.4
Willow wood charcoal	6	4	11	7.3 ± 0.7	35.4 ± 7.7
Acid washed bone black	6	3	5	3.5 ± 0.02	2.2 ± 0.4

RESULTS

In Table 1 are brought together the results on 147 cultures, arranged as percentages of the original egg counts. It is clear from the mean results here that the granular animal charcoal, willow wood charcoal, and the acid washed animal charcoal are significantly lower in effectiveness than the controls and the blood charcoal and bone black. About the same variability exists in all, except the acid washed charcoal, in which the results were remarkably constant and low. Or in culture terms, the apparently purified boneblack was consistently the poorest culture medium. As striking as the fact that the controls without any charcoal admixture were the best for culture purposes, is the fact that the blood charcoal exhibits not only better results than the bone black, but is barely different in biometric value, at 8.2 ± 2.3, from the controls themselves. These constants are all computed on the individual cultures irrespective of the stools cultured.

It was of interest to see whether the mean results from the various stools produced a different result. The data are not tabulated, but the

average results were within one per cent. of the means given in Table 1, with the coefficients of variation very much lower. Such a result indicates that the variable factors in hookworm cultures are not only between the cultures themselves, irrespective of stool source, but that any particular stool used may give somewhat different results from the one preceding or following it from the same person. The fact that the mean results are similar indicates quite clearly that to the extent which the stools studied are typical of all approximately neutral stools, unmodified 3 gram fecal cultures will furnish about 40 per cent., blood charcoal 32 per cent, and bone black 24 per cent. as many larvae as there are eggs introduced into the cultures. Or, if these particular percentages should not hold that cultures by these three methods will produce larvae in the ratio of 40 : 32 : 24.

The ratio of charcoal and feces which gave the most larvae was tested on the blood charcoal and bone black. Both sets of results are similar, and those of the bone black are given in Table 2 as a sample. From

TABLE 2.—*Experimental Cultures to Test Optimum Ratio of Feces and Bone Black in Hookworm Cultures*

Expt.	Ova in 3 Grams Feces	Larvae from Culture of 3 Grams Feces (Control)	Larvae Recovered from Cultures of 3 Grams Feces with Following Grams Charcoal							
			1.5	3.0	4.5	6.0	7.5	9.0	10.5	12.0
G	3600	2600	1760	980	1240	770	650	430	400	290
H	6600	2270	1380	1700	1560	1420	1160	560	420	700
L	7200	2280	1100	1930	1350	1860	330	270	60	60
M	6000	1440	1050	1210	970	550	1170	1150	820	500
Mean percentage larvae of eggs cultured		40.5	25.5	25.0	24.2	19.5	15.0	10.7	8.0	7.0

this table it is clear that mixtures of 3 grams stool with 1.5, 3.0 and 4.5 grams charcoal gave practically indistinguishable results, but that as the amount of charcoal was increased, there was a definitely smaller percentage of larvae produced. With between 3 and 4 times the amount of charcoal to the weight of feces, the percentage of larvae was reduced to a third or less of those secured with the closer to 1 : 1 ratios. The explanation for this is doubtless that inasmuch as the unmodified fecal cultures (controls) produced the most larvae, the charcoals "diluted" the food supply of the larvae, the more charcoal being present, the fewer larvae surviving.

It is a more or less common laboratory practice in making cultures for hookworm larvae with charcoal to use indiscriminate amounts of the two substances. These results indicate clearly, verifying Looss, that if charcoal is to be used, the larval crops are larger when approximately equal amounts of charcoal and feces are employed. Further, that with neutral stools there is no advantage in using charcoals at all.

These results also it should be pointed out, are strikingly different from those with acid stools derived from individuals on a tropical vegetarian diet, such as Stoll has demonstrated. In addition, both Stoll's earlier work, and my experiments, offer evidence from the two necessary angles, that while diet of the host is an important factor in the hookworm culture problem, the experimental situation is apparently just opposite to that reported by Stiles (1921): "Where much meat is eaten, as in northern climates and in city workers, the feces offer less favorable conditions for hookworm development than where little meat is eaten, as in southern climates and in rural districts."

SUMMARY

1. In a series of carefully standardized cultures for hookworm larvae, the best crops of larvae were secured with the nearly neutral human feces alone.

2. Blood charcoal is shown to be a superior substance to mix with stools for hookworm cultures, over bone black (animal charcoal).

3. Granular animal charcoal, willow wood charcoal and acid washed animal charcoal are distinctly inferior to the preceding.

4. Approximately equal portions of stool and charcoal are demonstrated to constitute the optimum mixture.

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AN EXTREME CASE OF OVER-PRODUCTION OF SHELL MATERIAL IN A TREMATODE*

H. W. Manter

In examining a blue-racer snake, *Coluber constrictor* (*flaviventris*) in Louisiana for parasites, twelve very interesting trematodes were found in the body cavity. These worms were actively moving about on the surface of the mesentery, stomach, and intestine. As the snake had been carefully opened and as no break could be found in the lung or intestine, it seems that the parasites did not owe their unusual location to mechanical injury produced during the examination. Moreover, the trematodes proved to belong to the genus Lechriorchis representatives of which have already been reported either wholly (*L. inermis*) or in part (*L. plesientera*) from the body cavity of snakes. A Renifer species has also been described from this habitat (Job, 1917). All except one of the present specimens showed a varying degree of abnormality in the condition of the female reproductive organs resulting from an over-production of shell and yolk material and leading to complete suppression of egg production, conversion of the entire ovary into a mass of shell and yolk substance, and an abnormal condition of the vitellaria themselves. Since the male organs remained normal, most of the trematodes had become physiologically males. A similar condition of over-production of shell material has been noted in a specimen of *Dicrocoelium lanceatum* by Goldschmidt (1909). The present forms, however, present the abnormality to such excessive degree that a brief account of the condition seems justified.

The trematodes were identified as *Lechriorchis validus* Nicoll (1911) with which they agree in all important points. Features showing considerable limits of variation were found to be (1) length of the ceca which reached only to or slightly beyond the testes; (2) length of the vitellaria which extend from somewhat beyond the acetabulum anteriorly to a point varying from anterior to the testes to slightly beyond them; and (3) posterior extent of the cirrus sac which scarcely reaches the middle of the acetabulum or extends to the posterior border of that organ. The form found agrees with Nicoll's description in size, shape, suckers, genital pore, digestive system, reproductive organs, and in egg size. No definite series of uterine swellings, however, could be seen in the descending limb of the uterus, and no definite branching of the excretory canals was evident although fine excretory tubules were

* Studies from the Zoological Laboratory, The University of Nebraska, No. 152.

frequent in the parenchyma. Nicoll's *L. validus* was obtained from the hog-nosed snake, *Heterodon contortrix* (*Heterodon platyrhinus*), with data lacking as to position in the host. Nicoll presumed that it was from the lungs, mouth, or esophagus. The region from which the host was obtained was not stated, but this snake is limited in range to North America. Considering the abnormality of the present specimens it may be that the hog-nosed snake (which is common in Louisiana) is the normal host of this species.

As has been stated, only one of the specimens collected was producing eggs and had a normal ovary. All the other specimens showed a diffusion of yolk or shell material resulting in a disorganization of the vitellaria, scattering of their products throughout the parenchyma, a complete filling and expansion of the ovary with such products, and an over-flow of particles and masses of the material into the uterus. The golden yellow to dark brown color of the substance thus distributed leaves little doubt that it is largely shell material produced by the vitellaria. In some regions, particularly within the ovary, yolk cells also were seen. The shell particles and masses produce a sharp contrast to the normal body structures stained with hematoxylin, a contrast impossible to illustrate in black and white figures. In early stages the disorganization appears in the vitellaria themselves, the yellowish-brown substance occurring free in isolated granules or globules of varying sizes. These products of the gland are spread and scattered throughout the parenchyma as if the vitellarian cells had burst like shells scattering shrapnel. One of the earliest effects is an ingression on the part of the material into the ovary. In the eleven specimens the ovary appeared entirely opaque and often swollen to several times its normal size (Figs. 2 and 3).

The size of the yellow or brown particles varies from minute granules to masses several times larger than the original yolk cells (Fig. 6). The larger masses are evidently accumulations of the smaller particles. A 5.2 mm. specimen showed only a very few normal egg cells in the ovary. These were located close to the wall of the organ, the remaining portion of the ovary being crowded with particles of the shell substance. In only one of the abnormal specimens were any eggs found in the uterus which, however, even when empty of eggs maintained a swollen expanded condition and usually contained considerable quantity of the shell substance. The few eggs found in one abnormal specimen varied greatly in size and shape, most being clearly abnormal, some being almost spherical (Fig. 7). It is interesting to note that the egg shell in these small eggs was extremely thin. Thus, the surplus shell material is not applied to any of the eggs. The yolk or shell substance was apparently being passed out from the body through the genital pore, the uterus acting as a sort of excretory canal. None of the substance was found in any

organ except the ovary, uterus, and parenchyma. None occurred in Laurer's canal. No other histological abnormality could be noted.

There are several possible explanations for this unusual condition. It may represent a natural senile condition and the breaking down of the vitellaria and dispersal of their products through the body might be caused by old age. One point in favor of this view is that the normal specimen was one of the smallest in size and at least one showing an intermediate condition was also intermediate in size (Figs. 1-3). It is known that as trematodes develop more and more past maturity the vitellaria tend to shrink in size as if exhausted. Sumwalt (1926) shows that in a very mature specimen of *L. plesientera* the vitellaria were very much reduced and had almost disappeared. A similar condition was seen in one of my abnormal specimens wherein flattened (normal) vitellaria were observed lateral to the shell material (Fig. 3). But in another specimen of equal size and fully as abnormal, regions of robust and healthy vitellaria could be discerned. Moreover, in the smallest specimen (5.2 mm.) the abnormality was extreme, not a single normal vitelline cell being visible, the ovary completely filled with the yellow-brown substance, the uterus with numerous masses of the material, and the parenchyma (especially dorsal to the ovary) well permeated with scattered particles and masses (Fig. 4). Hence, the degree of abnormality is not proportionate to increased body size as figures 1 to 3 might indicate.

In the next place the abnormality might be due to the possibility that the parasites were located in an abnormal habitat. Perhaps these trematodes normally inhabit the lungs and some injury led to their introduction into the body cavity. Sumwalt (1926) found the normal habitat of *L. plesientera* to be the lung, but collected two specimens from the body cavity in one host which was examined by him. In the present case no parasites were found in the lung. Again, it may be possible that the host was not the usual one for the parasite and this fact might have led to an active migration of the trematode from the lung into the body cavity.

No definite conclusions can be reached as to the correct interpretation of this condition of over-production of shell material. Furthur collections of the parasite in Louisiana may throw some light on the problem. The condition apparently represents a reaction due either to old age, or, more probably, to the effect of an abnormal host or an abnormal position within the host.

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Explanation of Plate XI

All drawings were made with the aid of a camera lucida. The projected scale has a value of 0.5 mm. in Figs. 1, 2, and 3; 0.1 mm. in Figs. 4 and 8; and 0.05 mm. in Figs. 5, 6, and 7.

Abbreviations: *e,* excretory system; *gp,* genital pore; *ov,* ovary; *sm,* shell material; *u,* uterus; *vt,* vitellaria.

Fig. 1.—Ventral view of normal specimen (*Lechriorchis validus*).

Fig. 2.—Ventral view of specimen showing early stage of abnormality.

Fig. 3.—Ventral view of specimen showing an extreme state of abnormality.

Fig. 4.—Sagittal section through an abnormal specimen showing shell material in parenchyma, uterus, and ovary.

Fig. 5.—Section through a portion of ovary flooded with shell material.

Fig. 6.—Enlarged view of shell material in the uterus, showing small particles collecting into masses.

Fig. 7.—Abnormal eggs from uterus.

Fig. 8.—Section through ovary partially flooded with the shell substance.

PLATE XI

NOTES ON DIFERENCES IN ACTIVITY AND RESISTANCE BETWEEN THE LARVAE OF *ANCYLOSTOMA DUODENALE* AND *NECATOR AMERICANUS* *

RUTH M. SVENSSON

In connection with studies (Svensson and Kessel, 1926) on the morphological differences between the larvae of *Ancylostoma duodenale* and those of *Necator americanus* a number of observations were made on the activity of these larvae and their resistance to adverse conditions, which indicate definite differences. Although the observations and experiments are of a preliminary nature, it seems worth while to publish them for the benefit of those working along similar lines, especially since it will not be possible for the author to continue work of this type.

Table 1 gives the results of a series of experiments conducted in Soochow, China, in the summer of 1924 to test the resistance of infective hookworm larvae to drying. Small glass jars were filled with dry sand and the surface moistened to 2.5 cm. in depth. Hookworm larvae, consisting of a mixture of about 272 *A. duodenale* to 18 *N. americanus*, which had been cultured 14 days before and had been somewhat attenuated by vertical migration, were placed on the surface of these units, with about 290 larvae per unit. The units were then left to dry in a shaded place in the laboratory. Every third day three units were isolated and the larvae counted and identified.† As shown in Table 1, the larvae of *Necator americanus* were less resistant to drying, all of them having died before the ninth day, while Ancylostoma larvae were still obtained from the jars after 20 days, only the isolations on the 22nd day being negative. Necator larvae also appeared to be less resistant to formalin than those of *A. duodenale*. A drop of water containing 70 *A. duodenale* larvae and 14 *Necator americanus* larvae were mixed on a slide with one drop of 5% formalin and a big cover glass placed over the preparation. All the larvae were observed at different intervals as shown in Table 2, and it was found that the larvae of *N. americanus* became motionless sooner than those of the other species.

Although the number of Necator larvae used in the experiments presented in Tables 1 and 2 is too small to warrant any definite conclu-

* The work on which this paper is based was carried out at the suggestion of Drs. W. W. Cort and N. R. Stoll of the China Hookworm Commission, to whom thanks are due for advice and help. I wish also to express my appreciation to Dr. J. F. Kessel for assistance in obtaining material for study and for helpful suggestions during the progress of the investigation.

† I am indebted to Dr. W. W. Cort for examining the last three sets of isolations.

sions as to the exact numerical relationships of the resistance in the two species of hookworm larvae, it seems evident that the results support the experiments showing a greater longivity for the larvae of *A. duodenale* under different temperatures (Svensson, 1925) in suggesting that the larvae of *N. americanus* are distinctly less resistant than those of the other species.

TABLE 1.—*Comparing the Reduction of A. duodenale and N. americanus Larvae in Drying Sand*

		Ancylostoma duodenale			Necator americanus		
Date	Day of Exposure	No. of Larvae Isolated		% Reduction	No. of Larvae Isolated		% Reduction
6/18	1st	290		0	18		0
6/20	3d	102 64 107	91	67	3 1 3	2.3	87
6/23	6th	98 116 92	102	62	1 2 1	1.3	93
6/27	9th	10 17 25	16	94	0 0 0	0	100
6/29	11th	46	46	83	0 0 0	0	
7/ 2	14th	74 39 24	45	83			
7/ 5	17th	8 20 6	11.3	95			
7/ 8	20th	2 2 1	1.7	99			
7/11	23d	0 0 0	0	100			

TABLE 2.—*A Comparison of the Reaction of A. duodenale and N. americanus to 5% Formalin*

	Ancylostoma duodenale: Motionless Larvae		Necator americanus: Motionless Larvae	
Time	Number	Per Cent	Number	Per Cent
After 15-22 min.	7	10	3	21.4
After 24-36 min.	20	28.6	8	57.1
After 36-50 min.	41	58.6	13	92.9
After 50-60 min.	43	64.3	14	100
After 60-75 min.	52	74.1	14	
After 80-98 min.	64	91.4		

After 98 minutes 6 *A. duodenale* larvae were still moving.

If further observations confirm this point of view it becomes necessary to explain how *N. americanus* is able to maintain itself in mixed infections of these parasites. Infection experiments carried out on two human volunteers suggest that the larvae of *N. americanus* have greater penetrating power than those of *A. duodenale* correlated perhaps with the presence in the larvae of the first species of a more effective boring spine (Cobb, 1923; Svensson and Kessel, 1926).

Two human volunteers of the same age, sex and race were infected, one with Necator larvae and the other with *A. duodenale* larvae. The Necator larvae were thirteen weeks old, thin and sluggish in their movements. The Ancylostoma larvae were two weeks old, well nourished, and active. In both cases twenty-five larvae were applied to the skin of the forearm by inverting the slides containing the larvae in a drop of water and leaving it in this position on the arm till the water had nearly dried. After thirty minutes, seventeen points of penetration were counted where the Necator larvae had been applied and forty-nine days later eggs appeared in the stools giving a count of 400 per gram with Stoll's egg-count method (Stoll, 1923). Where the Ancylostoma larvae had been applied only eight points of penetration were found after an hour, and stool examination for three months failed to reveal the presence of any hookworm ova.

Although these experiments cannot be regarded as conclusive, they strongly suggest that Necator larvae have greater power of penetration through the skin and of successfully reaching the intestine of the host than do the Ancylostoma larvae. The ability of the Necator larvae to penetrate readily has also been shown in experiments in Porto Rico mentioned by Cort (1925:87) where two human volunteers were infected with twenty-five and fifty larvae each and where the points of penetration when counted were twenty and forty respectively. The writer does not know of any similar experiments in the literature dealing with counted numbers of Ancylostoma larvae.

These preliminary experiments suggest the importance of further series of experiments comparing the larvae of *N. americanus* with those of *A. duodenale* in order to more clearly define the differences between these two species.

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HUMAN ASCARIS AS A HOUSEHOLD INFECTION

HAROLD W. BROWN *

Many records are available in the literature upon the subject of the epidemiology of ascariasis. On careful investigation of the data, however, they are found to be composed mainly of speculations and are not very conclusive. In countries where human excrement is used as crop fertilizer the evidence seems to be quite clear cut, at least in some cases, in incriminating vegetables thus fertilized and eaten raw or partially cooked. Isobe (1925) and others have demonstrated infective Ascaris ova on vegetables fertilized with human excrement. This mode of infection with Ascaris, although probably of great importance in countries where crops are so treated, is obviously of little importance in countries where pollution of the soil near growing crops is infrequent and where night soil is not used in agriculture.

In a number of countries where human excrement is not used on crops and the population is heavily infested with Ascaris, the drinking water supply has been suspected as the source of infection. Some of the older texts on helminthology (Cobbold, 1879: 247) advanced the belief that infections occur from drinking the water of small streams or other small bodies of water which have been polluted with human excrement. More recently Chandler (1925, 1926) has gathered evidence which indicates that in certain parts of India Ascaris infections are acquired from the drinking of water from "hulas" which are polluted by defecation upon their banks and even within them during the dry season. Even wind blown dust has been suggested as a source of Ascaris infection. In any case it appears that the epidemiology of ascariasis varies, as has been shown to be true for hookworm disease, within individual communities with one factor, namely soil pollution, common to all.

This paper embodies some attempts to shed light upon the etiology of Ascaris infestations in a section of the Republic of Panama where human excrement is not used as a crop fertilizer and the source of drinking water is in most cases free from any possibility of fecal pollution. Ascaris incidence in rural Panama varies from 40 to 90 per cent of the population.

METHODS

In as much as it was desired to exclude the factor of infections through polluted drinking water, families were chosen whose source of water supply seemed to be free from all danger of this possibility.

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It is a pleasure to convey my thanks to Dr. W. W. Cort and to Dr. N. R Stoll for their general direction of and interest in this work.

Several huts in Penonomé as well as a number of huts in the adjacent rural districts were chosen for this survey. These particular huts were chosen after routine egg counts (Stoll, 1923) had been made on the inhabitants and a heavy Ascaris infestation found present in several members of the family (Table 1). A careful survey was made of each

TABLE 1.—*Ascaris Ova Passed Per Day by Inhabitants of Huts Surveyed*

Town	House No.	Occupants: Age, Years	Occupants: Sex	Ascaris Ova per Gram Feces	Approximate Ova Passed per Day Based on 100 Grams Feces per Day	Total Ascaris Ova Passed by Household per Day
Penonome	A	30	F	No sample		
	A	5	F	104,600	10,460,000	
	A	7	F	149,200	14,920,000	25,380,000
Penonome	TA	2	M	9,000	900,000	
	TA	5	F	23,000	2,300,000	
	TA	8	M	64,000	6,400,000	
	TA	42	M	0	0	
	TA	30	F	11,550	1,155,000	10,755,000
Rio Grande	66	32	M	36,000	3,600,000	
	66	30	F	181,700	18,170,000	
	66	10	M	130,300	13,030,000	
	66	5	M	647,600	64,760,000	
	66	3	M	4,000	400,000	
	66	5 mo.	M	0	0	99,960,000
Rio Grande	4	35	M	3,000	300,000	
	4	25	F	85,200	8,520,000	
	4	2	F	20,000	2,000,000	10,820,000
Rio Grande	5	38	F	63,000	6,300,000	
	5	26	F	102,300	10,230,000	
	5	25	F	86,100	8,610,000	
	5	12	F	115,200	11,520,000	
	5	9	M	90,500	9,050,000	
	5	6	F	207,900	20,790,000	
	5	8	F	201,700	20,170,000	
	5	4	M	15,400	1,540,000	
	5	1	F	29,400	2,940,000	
	5	6	M	6,200	620,000	
	5	3	F	0	0	91,770,000
Cocle	8	77	M	100	10,000	
	8	42	F	0	0	
	8	18	F	29,600	2,960,000	2,970,000
Cocle	8	16	M	0	0	
	8	1	F	0	0	
	8	6	M	152,000	15,200,000	
	8	5	F	102,000	10,200,000	25,400,000
El Coco	11	19	M	No sample	0	
	11	38	F	12,600	1,260,000	
	11	18	M	29,500	2,950,000	
	11	14	F	0	0	
	11	6	F	191,400	19,140,000	
	11	3	M	141,600	14,160,000	
	11	12	F	189,000	18,900,000	56,410,000

hut and its premises including source of water supply, presence and use of latrine, presence of pigs and general living conditions of the people.

Sweepings were made from the dirt floors of the huts and also from the dooryards. The dust and soil thus collected was examined for ova by a method essentially like that outlined by the Caldwells (MSS). In the isolation of ova from the soil samples collected, the soil was first

thoroughly mixed by shaking it in a two quart jar. Then one to four 10 gram portions were used for each isolation. (See Table 2 for amount of soil collected and used in isolation.)

RESULTS

The general survey of the huts and the small plots of ground surrounding them furnished data mainly of a negative character. An inspection of sources of water supply showed that infection from that source was not likely. The inhabitants of the Penonomé huts which were examined obtained their water supply from the city water filter system and still they showed high Ascaris infestation. In fact, from a cross section survey of this town about 50% of the population was found to harbor Ascaris. All of the inhabitants of house 66 Rio Grande (Table 1) except a five months old baby had very high Ascaris egg counts, one boy aged five passing 64,760,000 Ascaris ova per day. Yet these people obtained their drinking water from a pump which was driven to a depth of 30 feet. The water supply of the other huts studied seemed equally free from danger of contamination with Ascaris ova. In only two of the rural locations studied were pigs kept and then in small enclosures. Had they been allowed to run at large they might have been a factor in the spread of human feces deposited about the yard (Chandler 1924). Also the presence of pig ascaris would have complicated the diagnosis of Ascaris ova from the soil isolations collected about the dooryards.

The latrine situation at the huts studied was very favorable at first sight. Each hut studied had a latrine, but it was not used by the children, who defecated either near the hut in the dooryard or in the hut upon the soil floor. The latter stools were usually swept out into the dooryard with a rude broom, leaving in most cases a small residue sticking to the floor. In the homes surveyed it was customary for the children and sometimes the adults to sit or squat on the floor while eating from a dish which was placed on the floor or on a small bench. This combination of living so close to the floor of their abode with the promicuous defecation upon it by the children would naturally be suspected as a source of infection by Ascaris. Isolations from floor soil bear this out.

The results of the isolations from the various situations are given in Table 2 in which the ova recovered are recorded according to their stage of development. Of the thirteen soil samples collected from hut floors and dooryards eleven were positive for Ascaris ova. Of isolations made from the floors of nine huts seven were positive for Ascaris ova. Both isolations made from the sweepings of one hut floor on two successive days were positive for not only Ascaris but also for Oxyuris ova. It is of interest that no Oxyuris ova were found in the stools of any of the

TABLE 2.—*Results of Isolation of Ova from Floors of Huts and Door Yards*

Town	House No.	Place of Soil Collection	Grams Soil Collected	Grams Used in Isolation	Ascaris Ova—Stages of Development							Other Ova
					One Cell	Early Morula	Late Morula	Tadpole	Motile	Degenerated	Total	
Penomome	A	Main hut	...	....	0	0	0	0	0	0	0	
Penomome	TA	First sweeping main hut	60	2-10	1	1	3	0	1	3	9	1 embryonated Oxyuris ova
Penomome	TA	Second sweeping main hut	55	2-10	2	1	2	0	1	2	8	4 embryonated Oxyuris ova
Rio Grande	66	Main hut	540	2-10	0	0	2	0	1	1	4	
Rio Grande	66	Cook hut	140	2-10	0	2	2	0	3	3	10	
Rio Grande	4	Main hut	565	1-10	0	1	0	0	0	0	1	
Rio Grande	4	Beneath eaves outside of hut	45	1-10	0	0	2	1	2	0	5	
Rio Grande	5	Main hut	120	2-10	1	1	1	0	0	0	3	
Rio Grange	5	Cook hut	255	2-10	1	2	2	0	0	0	5	
Coele	8	Cook hut	360	4-10	0	0	1	0	0	0	1	
El Coco	11	Main hut	330	2-10	0	0	0	0	0	0	0	
El Coco	11	Dooryard	830	2-10	6	5	0	0	3	3	14	
El Coco	11	Beneath eaves outside of hut	270	2-10	1,200 ova per gram of soil; ova only in 3 early and degenerate stages; none contained motile embryos							1 Trichuris ova isolated
Total					11	13	15	1	11	12	63	6

members of this family on the routine dilution egg counts. When later careful re-examinations were made a rather heavy Oxyuris infestation was found in a small girl of the family. In six of the thirteen places from which isolations were made Ascaris ova containing motile embryos were found.

The fact that Ascaris ova were present in quantities sufficient so that the rather gross technique employed detected them in the numbers it did, indicates that a rather large number of ova were actually present on the floors. Probably not over a tenth of the ova actually present in an isolation were transferred to the slide and examined. Granting this we find that from the 140 gram soil sample collected from the hut 66 Rio Grande about 700 ova were present and about 200 of them in the effective stage. Yet this soil sample probably represents only a small fraction of those that still remained upon the floor after sweeping. From Table 1 it will be seen that the very small residue of the children's stools left on the hut floors after the major part had been swept out contained large numbers of Ascaris ova. For example one tenth gram of the feces of the child aged five in hut 66 Rio Grande contained 64,760 Ascaris ova. This small amount of feces left upon the floor, and no doubt a quantity even greater then this is often left, would be sufficient to produce a very heavy Ascaris infection center within the hut.

As will be seen from Table 1 although no ova were isolated from the floor of hut 11, El Coco, ova were found in the dooryards. A spot under the eves in back of the hut which was in the direct sunlight most of the day was pointed out by the children as their usual site of defecation. These two children were passing a total of 23,000,000 Ascaris ova per day. A soil sample isolation showed about 1,200 Ascaris ova per gram to be present here. None of the ova were beyond the morula stage while most of them were plainly degenerated. There is here what might have been an ideal situation for a focus of Ascaris infection for with the pile up of pollution in this spot one would have expected it to be teeming with infective ova. The fact that all the ova were degenerate except those just recently passed is in accordance with the results of the writer (Brown, MSS) from *planting stools upon sandy soil in the direct sunlight.* In cultures thus made the Ascaris ova all degenerated in from two to three weeks.

The following experiment was made to ascertain whether or not the Ascaris ova which remained on the floor of the hut after the major portion of the stool had been swept out would develop to the infective stage. Small amounts of feces containing Ascaras ova were smeared on an earthen floor similar to those of the huts studied. An isolation from the floor 14 days after planting showed 15 per cent. of the ova to contain motile embryos. Seven days later or 23 days from the planting of the feces 23 per cent. of the ova were in this stage.

DISCUSSION

The finding of Ascaris ova on such a high percentage of the floors of the huts and about the dooryards suggests that this is an important source of infection of the people. Khalil (1924) working in Egypt demonstrated Ascaris ova in the sweeping of the dooryard and floor of one hut. Two of the ova contained motile embryos and he concludes this to be a probable source of infection. The additional evidence that it is usually the children who are more likely to harbor Ascaris is in keeping with their close association with the floors of the huts and ground in the dooryards.

That Ascaris ova can develop to the infective stage on the floors of huts does not exclude the possibility that they may also find their way there from pollution about the yard. In fact it has been shown by other workers that helminth ova are sometimes transported some distance upon the bare feet or shoes. By this method of contamination, although no actual fecal pollution by children of the floors took place, ova would still be present on the hut floors. The fact that Ascaris and Oxyuris ova were present in isolations from sweepings of the same floor on two successive days demonstrates that the haphazard sweeping the hut floors probably does not free them from ova entirely.

CONCLUSIONS

1. Ascaris ova were found to be present in the soil sweepings of dooryards and hut floors in six of seven situations about Penonomé, Panama.

2. It seems very probable in view of the close association of the inhabitants with their hut floors and dooryards that these are a source of Ascaris infection of considerable importance.

3. In eleven out of thirteen soil samples from hut floors and dooryards Ascaris ova were present.

4. The Ascaris ova were in all stages of development, six of the thirteen soil samples containing ova in the motile embryo stage.

5. Oxyuris ova were isolated from the floor of one hut and upon careful examination a child living there was found infested with this parasite.

6. Ova passed in feces upon huts floors, as is the custom of the children, develop to the motile embryo stage in 14 days.

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SOCIETY PROCEEDINGS

SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS, DECEMBER 28-30, 1926, PHILADELPHIA, PA.

GENERAL DISCUSSION OF MEETING

The second annual meeting of the American Society of Parasitologists was well attended and very successful. About 75 were present out of a total membership of 392. This number is about one-fourth of those resident in the United States. The sessions were also well attended by other interested workers. A feature of the program was a very interesting and splendidly illustrated address on Piroplasms by Professor G. H. F. Nuttall of Cambridge University. Dr. Nuttall discussed representative life cycles in this group. In addition to an analysis of present knowledge, he emphasized especially the many interesting problems that remain unsolved. He ended with a plea to American parasitologists to devote more attention to researches on this group of organisms. Dr. Nuttall was able to attend most of the sessions of the Society and took part in the discussions. His presence added very greatly to the meetings.

The retiring presidential address by Dr. C. W. Stiles was given Wednesday morning at a joint meeting with the Zoologists. Dr. Stiles spoke on certain problems in nomenclature under the title, "Underlying Factors in the Confusion in Nomenclature, with a Definite, Practical Suggestion for the Future." His thesis was that most of the confusion in present zoological nomenclature is due to the ignoring by writers of a few of the simplest rules. His suggestion for future improvement consisted of a plea for some formal instruction in zoological laboratories in the principles and rules of nomenclature. It was pointed out that only a comparatively small amount of time would be necessary for such instruction, and that there was no more excuse in the training of a zoologist for neglecting instruction in nomenclature, which was designated as the "Grammar of Zoology," than for the omission of grammar in the ordinary school curriculum.

The contributed papers on the program were representative of the work in parasitology which is being done in the United States. A number of unusually important contributions on a variety of phases of the subject were included. As a whole the papers showed careful preparation, were well presented and stimulated interesting discussion. Forty-five papers were listed on the program of the Society, of which thirty-four were presented by the authors and eleven read by title. Seventeen were in Protozoology, twenty-seven in Helminthology and one in Medical Entomology. Only about one fourth were on the systematic-morphological phases of the subject. Most of the others were experimental in nature, dealing with immunity, life-cycle relations or the economic and medical aspects of the subject. This shows a new emphasis in American Parasitology. Such a program ten years ago would have been very largely dominated by papers of the strictly systematical and morphological type. The practical relation of parasitological researches was shown by the fact that about half of the papers dealt either directly or indirectly with human parasitology and eight more had to do with parasites of animals of economic importance to man.

ABSTRACTS OF CONTRIBUTED PAPERS

Thelazia Infections in China. Ernest Carroll Faust, Peking Union Medical College.

Various species of Thelazia have been described from the eye of mammals and birds. Human cases have been reported only from China (Stuckey, 1917;

Trimble, 1917). On the basis of Houghton's description (1917) of the Stuckey material Leiper (1917) believed the human specimens belonged to the species *Thelazia callipaeda,* originally described by Railleit and Henry (1910). The writer has studied (1) the original Stuckey material, both from man and from the dog, (2) worms recently obtained from both eyes of a foreign dog in Peking, (3) a single female worm from the eye of a Peking laboratory rabbit, and (4) worms secured from the eye of a cow near Soochow. Examination of the material from man, dog and rabbit shows it to belong to the species *Thelazia callipaeda,* while that from the cow is *Thelazia rhodesi.* These findings therefore confirm Leiper's belief that the human worms belong to the same species as those of the dog and in addition indicate that there is considerable latitude in host selection by the parasite. The record of *T. rhodesi* from China is also new. The material from man and the dog consists of both female and male specimens, thus making it possible to describe for the first time the males of this species. These male specimens consistently have one very short and one very long copulatory spicule, five paired and one unpaired precloacal papillae and two pairs of postcloacal papillae. The material also furnishes an opportunity for a more accurate description of the buccal capsule than was obtained by Railliet and Henry from their original material. From the ventral aspect this structure appears as a triangular object, with ventral, anterior (dorsal), and lateral shanks, the latter two being continuous dorsally into a capsular hood. Since no larvae were found in the peripheral blood of the infected dog recently observed, which harbored altogether forty-two worms, and since larvae were abundant in the conjunctival fluid, it is suggested that the infection is probably transmitted by a fly or some other insect which might serve as intermediate host and transfer the infection by direct contact.

Parasites as a Limiting Factor in Ohio Fish Hatcheries. Ralph V. Bangham, College of Wooster.

A preliminary survey of parasites as affecting fish in state hatcheries was made during the summer of 1926. Only a small per cent of the bass spawned successfully this season. Examination showed that this was largely due to infestation by larval *Proteocephalus ambloplitis* (Leidy). In one Ohio hatchery with 150 small-mouth breeders no fish spawned. The visceral organs and membranes of these fish were very heavily parasitized with these larval cestodes. They were found encysted near the surface of the liver, spleen, and reproductive organs and also causing fibrous adhesions and hemorrhagic areas in the mesenteries. The ovaries had from 10-60 of these worms coiled between the eggs near the surface. The eggs near the worms appeared to be degenerating and there were hemorrhagic areas near each worm. These worms were also found in the testes, which appeared knotted and with much of the normal tissue degenerate and fibrous. An ecto-parasite, *Ichthyophthirius multifiliis* (Fouquet), caused much damage to young large- and small-mouth bass in two hatcheries. A trematode, *Gyrodactylus* sp., caused damage in two of the largest hatcheries to goldfish, catfish and blue gills. In one hatchery the body surface of these species of fish was attacked by a mixed infestation of this trematode and Ichthyophthirius. Parasites were found chiefly in the region of the fins. Another trematode which may limit the growth of young large-mouth bass is the gill parasite *Ancyrocephalus* sp. This form was found on young large-mouth bass in every hatchery, attached to the inside of the mouth and to the gills.

An Extreme Case of Over-production of Shell Material in a Trematode. H. W. Manter, University of Nebraska.

Eleven of twelve specimens of *Lechriorchis validus* collected from the body cavity of a blue-racer snake in Louisiana showed an excessive over-production of shell and yolk material. These surplus products, varying from small particles to large masses, completely fill and expand the ovary, flood whole regions of the parenchyma, and overflow into the uterus. Egg production is thus completely suppressed although the male organs remain normal. None of the surplus material was found in Laurer's canal. The condition may possibly be explained by senility. More probably it is associated with an abnormal host or an abnormal location within the host.

Development of *Sarcocystis tenella* in the White Rat. John W. Scott, University of Wyoming.

An attempt has been made to use the white rat in working out the life history of *Sarcocystis tenella.* Young rats after fasting fifteen to twenty hours were fed two or more Balbiania, each probably containing millions of spores. The rats were killed at intervals; portions of the intestine were fixed for sectioning; parts were used for smears of the contents, and a third series of parts discarded. Progress of the parasite along the alimentary canal can thus be observed. For example, in one rat two hours after feeding empty spore cases were found from nineteen to twenty-three inches below the stomach. Some amebulae escape from the spores in the stomach or very soon after entering the intestine. Sarcotoxin is set free and causes extensive destruction of the villi; a serous exudate from the injured villi adds to the contents of the intestine. Amebulae are most frequently found along the injured surfaces; a number penetrate the epithelial cells, and undergo some development. There are two types of ambulae, probably male and female, but their later development has not been followed. Whether they enter the region of the lymph spaces, changed in staining reaction, or are thrown off with the epithelium and lost with the feces, is as yet uncertain. Several rats fed infected muscle (not Balbiania) and killed six to twenty weeks after feeding showed no sarcocysts in the muscle. From this it would seem that *Sarcocystis tenella* cannot complete its life history in the white rat.

Species and Strains of Coccidia in Poultry. E. E. Tyzzer, Harvard Medical School.

Eimeria oöcysts from chickens obtained from different sources show marked differences in pathogenicity. The acute coccidiosis of the chicken, marked by extensive and often fatal hemorrhage into the caeca and lower intestinal tract, is believed to be due to a distinct species to which the name *Eimeria tenella* of Railliet and Lucet is perhaps applicable. Its differentiation is based on the relatively huge size of the schizonts, the large size of the merozoites, deep penetration of the tissues, pathogenicity, localization and type of development. Coccidiosis in chickens appears commonly to represent a mixed infection, for it is possible to isolate strains if Eimeria which produce only small schizonts and merozoites, which develop superficially in the epithelium of the small intestine without invading the caeca or producing hemorrhage. Such strains of Eimeria have been isolated which are practically innocuous, having little effect on the health and growth of young chickens. Since Rivolta and Silvestrini made no mention of hemorrhage in the coccidiosis which they describe, the older name *Eimeria avium,* if available, may be applied to the non-pathogenic type found in the chicken. It has been found impossible to infect young chickens with Eimeria oöcysts obtained from turkeys and likewise impossible to transmit either the pathogenic or non-pathogenic strains of chicken coccidia to young turkeys. These results confirm Johnson's suggestion that the form encountered in the turkey may represent a distinct species and for this the name *Eimeria meleagridis* n. sp. is proposed.

The Thermal Death Point of Trypanosomes and a Preliminary Report on the Immunization against Trypanosomiasis. Damaso de Rivas, Medical School, University of Pennsylvania.

Experiments *in vitro* made with the blood of rats infected with *Trypanosoma lewisi* and *T. equinum,* showed that in the parasitized blood when kept at low temperature, not over 5°C. in sealed capillary tubes, the parasites live for from 10 to 18 days, but their life was much shortened, when kept at room temperature or in the incubator at 37°-38°C. When the same experiments were repeated at 45°C. the trypanosomes were killed in 5 to 15 minutes and the subcutaneous injection of the heated blood into fresh normal rats failed to produce infection. Based on these observations, the possibility was next considered of applying these results in the immunization against trypanosomiasis by a previous attenuation of the parasite by means of the action of heat. After repeated experiments it was found that the immunization against trypanosomiasis in rats was readily obtained

by the application of subcutaneous injection of inactivated trypanosomes as follows: First injection with parasitized blood heated at 45°C. for 15 minutes. Second injection, one week later, with parasitized blood heated at 45°C. for 10 minutes and a third injection with parasitized blood heated for 5 minutes at 45°C.

Chemical Change in Red Blood Corpuscles Parasitized by Plasmodia as shown by Golgi's Gold Chloride Staining Method. Ernest Hartman, University of Illinois.

Golgi (1919 and 1920) reported the presence of a new body, "reticulofibrillar apparatus," in both nucleated and non-nucleated blood cells. In the non-nucleated red cells this body occupies a central position and is distorted as the corpuscle is distorted. In nucleated red and in white cells the body surrounds the nucleus. The exact nature of this body is undetermined but the regularity of its occurrence leaves no doubt that the gold is deposited on the basis of a real structure within the cell. Corpuscles infected with *Plasmodium vivax* and with *P. praecox* show a reduced body when the parasite is small and very little or no "reticulofibrillar apparatus" when the parasite is large. Gold is not deposited to any great extent in cells parasitized by large organisms. In birds malaria especially the effect of the parasite on this apparatus can be plainly seen where the side of the nucleus near the parasite often shows a smaller deposit of gold than does the opposite side. The Plasmodia cause a chemical change in the parasitized cells such that the gold is not deposited as it is in the normal cells. Until the chemistry of this depositing of gold is better understood it is impossible to determine the chemical change due to the parasite. This phenomenon seems to favor the side of an old argument which is now taken by most malariolgists; namely: that the parasite of human malaria is located within rather than on the corpuscle.

A Precipitin Test in Human Malaria. William H. Taliaferro, Lucy Graves Taliaferro and Anna B. Fisher, University of Chicago.

In attempting to find a precipitin test in human malaria 1605 tests with 37 different antigens were made on 535 persons at the Tela Hospital of the United Fruit Company in Honduras. Ring tests were carried out in small tubes, and the positive results were listed as +++, ++, and + according to the amount of precipitate. The best antigen was prepared by running heavily infected placentas through a meat grinder, extracting in ether, digesting the ether insoluble portion in Coca's solution, filtering through hard filter paper and using the clear filtrate. Samples of sera from 86 persons were tested against this antigen. Of these, 54 were found infected in either thick or thin blood films and gave the following results: 10 were +++; 21 were ++; 14 were +; 2 were doubtful; and 7 were negative. The remaining 32 persons were negative by the thick film method and gave the following results; 2 were ++; 4 were +; 1 was doubtful and 25 were negative. This is a very high correlation and indicates that a precipitin test can be devised for the diagnosis of malaria.

The Acquisition of an Active Immunity by Dogs and Cats to Infection with *Strongyloides stercoralis.* J. H. Sandground, Harvard Medical School.

In the course of studies on the specific relationship existing between different species of the genus Strongyloides and their various hosts, it was found that the human species, *S. stercoralis,* could be established in many dogs apparently as a permanent parasite. In one dog, the initial infection disappeared spontaneously in the course of a few months and, although several attempts were made subsequently, the animal became refractory to reinfection. In other dogs, the initial infection tends to decrease in its intensity within the course of a few weeks but is not entirely overcome. In such animals attempts to increase the infection by superimposing a second infection are not effective. Nine cats of various ages were subjected to infection with the same parasite. The prepatent period before the parasites attain sexual maturity is, in general, appreciably longer in cats than in dogs and, in all cases, the infection spontaneously disappears in a short time. The animal is thereafter completely refractory to reinfection. Also in cats, it is only occasionally possible to superimpose a second infection with the parasite. The resistance to infection in these instances is independent of the age of the host.

A Well-Defined Group of Furcocercous Cercariae resembling Schistosome Larvae in Certain Respects. Harry M. Miller, Jr., Washington University, St. Louis.

Recent descriptions of four cercariae make it possible to characterize more precisely the Elvae group (Miller, 1923 and in press), which has eight members. These cercariae come from widely separated areas: Europe, South Africa, India, China and the United States. They are large apharyngeal distomes with spindle shaped bodies in which there is a highly specialized anterior penetrating organ. The furcae are not as sharply constricted from the tail stem as in schistosome larvae, and in length are never less than one half that of the tail stem. In this relation of furcal length to tail stem length the members of the Elvae group more closely resemble the pharyngeal longifurcate larvae, but in all other respects they are more similar to the schistosomes. An alimentary canal of the same type as found in the schistosomes is present, except that there are short finger-like ceca. Deeply pigmented, well-defined, relatively small eye spots are constantly present. The penetration glands are large and the ducts heavy. The excretory system, which is similar in the five forms for which it has been studied, is quite similar to that of the schistosomes; there are five pairs of body flame cells and one tail stem pair in two cercariae; and an additional posterior body pair in three (probably four) larvae. The parthenitae are elongate sporocysts, never rediae. Nothing is known of post-larval development; encystment has not been reported.

Preliminary Report upon the Acanthocephala from Fishes of China. H. J. Van Cleave, University of Illinois.

Through the extensive collections of Dr. E. C. Faust, I have had access to unusually large collections of Acanthocephala from China. Not a single species of these parasites has been recorded from the fishes of China. In the collections at hand, the Acanthocephala taken from fishes represent a number of previously unknown genera. The systematic relationships of these new forms will be discussed.

An Interesting Sand Fly Outbreak in Texas. F. C. Bishopp, Bureau of Entomology, U. S. Department of Agriculture.

A species of sand fly, *Culicoides variipennis,* which normally breeds in salt water along the coast has established itself in the creeks in central Texas which during recent years have been made salty by the discharge of salt water into them from the oil fields. The insect, which is a vicious biter, has become a serious pest to man and live stock in the vicinity of these salty streams, where it is now breeding in great numbers. The character of injury, breeding habits, and life history are outlined.

Immunity to Superinfection with *Plasmodium relictum* in the canary. William H. Taliaferro and Lucy Graves Taliaferro, University of Chicago.

Previous work has shown that the low grade chronic infection, and probably the latent period which occurs in canaries after the initial crisis, is associated with some mechanism which destroys the parasites as fast as they are formed, but which does not alter the rate of their asexual reproduction. If large quantities of parasites are injected intravenously into a bird during the latent period of its original infection they are killed in from 15 minutes to several hours; whereas if the parasites are similarly injected into uninfected birds; they live and immediately begin a new infection. This not only demonstrates that there is some condition during the latent period which is quickly lethal to the parasites, but shows that the parasites do not have to wait until they emerge from the red blood cell (sporulation) and come into contact with the serum to be killed. On the hypothesis that the lethal agent is an antibody numerous attempts have been made to transfer it passively from bird to bird (by trying both protective and *in vivo* lytic experiments) but so far all of these have failed. The lethal agent may be some change in concentration of the simpler blood constituents.

Observations and Experiments on a Tritrichomonas of Man with the Ability to Grow and Multiply Indefinitely in Feces Diluted with Tapwater and in Cold-blooded Hosts. L. R. Cleveland, Harvard Medical School.

This Tritrichomonas, studied for two years, was first observed in a stool kept at room temperature in a gallon of tapwater. Nine out of ten stools collected from the same person (male) on different days and diluted with tapwater yielded the organism, together with many free-living protozoa from which it was separated by anaerobic cultivation in serum-saline-citrate medium at 36°C. It was never found before 15 days and was not abundant before 20 to 25 days; it remained abundant for a year or more provided tapwater was added to prevent drying. Heated stools were always negative. Eight unheated stools collected a year later were positive. Microscopic examinations of the feces were negative; a single Trichomonas-like organism was seen twice in mouth scrapings. The organism grows in serum-saline medium from —2°C. to 36°C.; practically any fluid which supports bacterial growth supports it; heat-killed bacteria in saline support it. Red blood cells (any particular matter) are eaten in abundance when bacterial food is scarce. Twenty tadpoles were placed in oxygen and freed of their Tritrichomonas (morphologically distinct from that of man) and then fed the stool cultures of Tritrichomonas from man. These were killed and examined at intervals up to 100 days and all were infected. Ten frogs were treated in the same manner and the last one was heavily infected after four months. All attempts to grow Trichomonas and Pentatrichomonas from man in tapwater-diluted feces failed, but the frog Tritrichomonas grows abundantly.

A New Trematode, *Vasatrema amydae*, n. g., n. sp., from the Vascular System of the Soft Shelled Turtle Amyda. Horace W. Stunkard, New York University.

Four specimens were found in the circulatory systems of *Amyda ferox* taken in Florida and many others in the vascular system of *Amyda spinifera* from Indiana. They may contract into an oval mass or become so attenuated as to be almost filiform. Fixed specimens measure from 1.2 to 3 mm. in length, 0.07 to 0.35 in width, and from 0.04 to 0.1 mm. in thickness. The musculature is delicate and the suckers weakly developed. The acetabulum is slightly protrusible but not stalked, situated about one-fourth of the body length from the anterior end. It measures from 0.04 to 0.13 mm. in diameter. The oral sucker is usually slightly longer than broad, 0.035 to 0.075 mm. in diameter. Pharynx absent, posterior half of eosophagus bears several dilations. Digestive tract bifurcates immediately anterior to acetabulum, ceca extend to caudal one-eighth of body. Ovary oval, 0.03 to 0.07 mm. in diameter, situated slightly anterior to middle of body. The oviduct passes back to the ootype and uterine duct leads forward to genital pore, Laurer's canal present. Testis elongate, coiled, extending from the center of the body backward between the digestive ceca. Seminal vesicle large, cirrus sac small. Genital pore ventral, sinistral, between the ovary and acetabulum. Vitellaria lateral, extend from ovary to caudal ends of ceca. Eggs oval, very large, discharged singly, miracidia with prominent eye spots. This form is included in the sub-family Hapalotreminae, family Spirorchidae and presents further knowledge concerning the blood infesting trematodes.

On the Specific Identity of *Amphistomum bicaudatum* Poirier and *Cladorchis gigas* MacCallum. Horace W. Stunkard, New York University.

Poirier (1908) in the Compte Rendu of the 37th session of the French Association for the Advancement of Science held at Clermont-Ferrand under the title "Trematodes parasites de l'elephant d'Afrique," published the description of *Amphistomum bicaudatum*. In 1917 MacCallum published the description of a similar fluke from the same host naming the parasite *Cladorchis gigas*. Travossos (1921) created the genus Brumptia to contain MacCallum's species. Maplestone (1923) made corrections and additions to MacCallum's description. Comparison of the description of Poirier with those of MacCallum and Maplestone indicated that the two forms are identical and more recently through the kindness of Dr. Roubaud of the Pasteur Institute, Paris, I have had the opportunity to compare Poirier's specimens with those of MacCallum. This examination proves that the worms belong to the same species for which the correct name now becomes *Brumptia bicaudatum* (Poirier) Stunkard. Discussing this form in my recent synopsis of the Paramphistomidae, I named it as type of a distinct sub-family Brumptinae.

Development of *Belascaris marginata* in Prenatal Infestation. Donald L. Augustine, Medical School and School of Public Health, Harvard University.

Belascaris marginata in prenatal infestation remains in the liver of the dog embryos showing little or no development beyond the first larval stage. One day after birth of the young dogs, the larvae may be found in the lungs, and from five to six days later in the intestine. The development of the larvae is rapid after leaving the liver of the newly born dog. By the time they were encountered in the intestine they were found to have attained from four to six times the length of the liver and lung forms. They become sexually mature with ova appearing in the feces about one month after birth of the puppies. The development of the larvae which remain in the pregnant bitch is not retarded as in her unborn young. Pregnant animals over one and one-half years old, inoculated with embryonated ova of *B. marginata* one month after coition, showed at autopsy no worms in the liver, lungs or intestine, while as many as sixty worms were found in the intestine of one of her puppies. Animals less than one and one-half years of age, similarly inoculated, showed at autopsy a few worms in the intestine. In each of her puppies, however, where intestinal forms were present, the infestation was always several times heavier than that of the mother dog. It therefore appears that her age is a factor in the number of worms becoming established in the puppies as well as in herself.

Host-Parasite Relations between the Dog and the Hookworm *Ancylostoma caninum*. C. A. Herrick, School of Hygiene and Public Health, Johns Hopkins University.

Some of the relations between the host and the dog hookworm *Ancylostoma caninum* are being studied by determining the egg laying capacity of the female worms. By the use of accurate methods for the detection and counting of hookworm eggs in feces one can determine not only the time required for the female worms to become sexually mature but also the course of the infection throughout the life of the worms or for any desired period. By using these diagnostic and counting methods natural and experimental hookworm infections have been studied in different aged street dogs, dogs born in the laboratory and those bred and raised in cages. In about ten days following an infection, diagnosis is made for the presence of hookworm eggs. After the first eggs are found in the feces, daily dilution eggs counts are made on the total fecal samples and the number of eggs per gram and the eggs per day are recorded for each dog. The data so far obtained indicate that (1) the average time required for the worm to reach sexual maturity increases as the dogs grow older; (2) the per cent of the hookworm larvae that are able to grow to maturity varies inversely as the age of the host. The type of infection is, however, much the same in all cases: the production of eggs by the hookworms rises more or less rapidly to a peak and then falls to a level which may remain fairly constant for several months; (3) regardless of the age of the host or the number of larvae given some fail to develop even after several weeks; (4) dogs, which are harboring hookworms, seem to be more resistant to infection than are non-parasitized dogs or dogs previously parasitized by hookworm but freed from worms by an anthelmintic before reinfection.

Resistance to Parasitism Affected by the Fat-Soluble Vitamin A. J. E. Ackert, Marian L. Fisher and Naomi B. Zimmerman, Kansas State Agricultural College.

Thrifty chicks one month or more old show resistance to the intestinal roundworm, *Ascardia lineata* (Schneider) by eliminating young worms from their bodies and by retarding the growth of the remaining parasites. The high incidence of this parasite in nature indicates that factors in the chick's diet or environment may lower its resistance to the parasites. As the food of early spring chicks is likely to be deficient in the fat-soluble vitamin A, tests were made to ascertain if their resistance to parasitism is lowered when given a diet inadequate for that vitamin. Three groups of pure bred chicks from the same hatches were placed on the following diets; the first group on a synthetic diet inadequate for vitamin A, the second on the same diet except for the addition of vitamin A, and the third group on a natural diet containing vitamin A. All chickens were supplied with proteins, fats, carbohydrates, minerals and vitamins B and D. When the chickens

had been kept on these diets for a period of three weeks, they were parasitized by each chick being given approximately the same number of embryonated eggs of *A. lineata.* The experiment was then continued for three weeks when all chicks were autopsied. In five of such tests using ten chicks per group the results show that those groups of chicks whose diet was deficient in vitamin A had significantly more parasites and larger parasites than did those groups of chicks whose diet contained this vitamin in abundance.

Confirmatory Evidence that Habronema larvae are not the Etiological Factor in Bursattee. Stanley B. Freeborn, George H. Hart and Carroll E. Howell, University of California.

In 1860, Ercolani discovered a larval worm in the skin lesions of horses known as bursattee, summer sores, granular dermatitis, etc., which he named *Trichina uncinata.* Rivolta named a nematode found in the lesions *Dermofilaria irritans* and for many years the etiological factor has been considered to be *"Filaria irritans."* Raillet basing his contentions on the findings of Descauzaux, in Brazil, and the elucidation of the life history of Habronema by Ransom voiced the opinion, which has been widely accepted, that this type of lesion was caused by the presence of larval worms of the genus Habronema. In 1919, we attempted to demonstrate Habronema larvae in 50 adult house flies captured on affected animals at Davis. This attempt yielded only negative results in spite of the fact that Ransom had found them so numerous in "wild" flies that he proposed to diagnose horse manure-bred flies by their presence. This year, influenced by the sporadic occurrence of bursattee in the college horses, we attempted to determine definitely if Habronema were present. Autopsies of two animals and the record of all past autopsies made by the Veterinary Science division were negative for Habronema, as were the dissections of 250 house flies bred in the college barns, thirty-four were also negative for Habronema embryos. In addition, microscopic examinations of scrapings from all stages of lesions and a careful study of histological sections failed to show parasites, eosinophilia, or the characteristic pictures described by other writers as typical of worm infested lesions. With these findings in view, we believe we are correct in reporting a situation where bursattee sores are numerous and Habronema entirely absent. On this basis, we assume that the infestation of bursattee lesions by Habronema is accidental and does not constitute the etiological factor.

The Intestinal Amoebae of the Cockroach. Catherine L. T. Lucas, London School of Hygiene and Tropical Medicine.

Endamoeba blattae (Bütschi, 1878) Leidy, 1879, exhibits the following features, which together distinguish it from all members of the genus Entamoeba, and probably indicate generic distinction:—(1) in size it ranges up to 0.13 mm. in diameter; (2) it has a steady flowing movement without distinct pseudopodia; (3) the nuclear membrane appears like a cyst wall and is 0.001 mm. thick; (4) there is no compact karyosome; in adult forms the chromatin is often entirely in the peripheral zone; (5) repeated nuclear division occurs before encystation so that free forms are found with 8 or more nuclei; (6) ripe cysts have 40-50 nuclei. While resemblances exist between *E. blattae* and such forms as *E. ranarum* and *E. testudinis* it is possible that phylogenetically it is more nearly related to certain of the free amoebae. Certain features of nuclear division, which has been studied in living individuals and observed in stained material will be described. Two new amoebic species have been discovered in the cockroach, an *Entamoeba* sp. and *Endolimax blattae,* n. sp.

Studies on the Rate of Development in Nature of *Ascaris lumbricoides* and *Trichuris trichiura* ova with some Notes on the Epidemiology of these Species in Panama. Harold W. Brown, School of Hygiene and Public Health, Johns Hopkins University.

Experimental studies were made to ascertain the length of time necessary for the ova of *A. lumbricoides* and *T. trichiura* to attain the motile embryo stage when passed in stools on the soil. Feces containing the ova of these two forms were planted on sand, loam, clay and humus soils in both the sun and the shade and periodic isolations made from them to obtain the stages of development of the

ova. Practically all of the cultures thus made contained Ascaris ova in the motile stage in 15 days, while some cultures contained Trichuris ova in this stage in 21 days. The development of the ova was to some extent controlled by the type of soil they had been placed upon. All ova in the sand soil culture in the sun degenerated before reaching the infective stage while the ova in the humus soil in the shade were three weeks behind these of the other soil cultures in the shade. A survey of the temperatures and rainfall of a number of states in the Southern United States indicates that during the four summer months they have climatic conditions very similar to those found in Panama during the course of these experiments. By means of a flotation technique Ascaris ova were recovered from the sweepings of the floors and dooryards of a number of huts in Panama. Infective ova being present in half of the cases. Ascaris and Oxyuris ova were found in the sweepings for two consecutive days from one hut. At least a part of the ova recovered from the mud floors were from the stools of the smaller children who defecate there and in brushing the floor a residue is left. Ova of Ascaris were found to develop to the motile embryo stage when deposited upon the mud floors of the huts in 14 days.

Excystation of *Iodamoeba williamsi* in Vitro and in Vivo. Septima C. Smith, School of Hygiene and Public Health, Johns Hopkins University.

The process of the escape of the motile human entozoic amoeba, *Iodamoeba williamsi,* from its enveloping cyst membranes has never been described. The whole process, from the earliest activity of the amoeba inside the cyst wall to its complete exit, leaving the empty cyst membrane behind, has been observed by the writer many times, in washed cysts confined under a cover glass. The conditions necessary for excystation have been studied and the process can be brought about at will by supplying the proper stimuli. Excystation occurs when washed cysts are injected into the stomach of the guinea-pig. It has been noted in the jejunum and ileum. The excysted amoebae apparently do not find the guinea-pig intestine a favorable habitat since they cannot be recovered later.

Further Studies on the Causation of Creeping Eruption. W. E. Dove and G. F. White, U. S. Bureau of Entomology.

Creeping eruption is a human skin disease due to an invasion of the skin and the migration therein by a nematode larva. Earlier it was generally supposed that the parasite causing creeping eruption lesions of the skin was a fly larva, especially that of Gastrophilus. There are indeed creeping diseases of the skin far less frequently encountered, however, that are caused by fly larvae. Kirby-Smith, Dove and White have demonstrated that one of these creeping diseases which they have designated "Creeping Eruption" is caused by a nematode larva. Further studies have shown that nematode larvae cultured from the feces of cats and dogs are concerned in the causation of this disease. An autopsy on twenty-seven street dogs in Jacksonville, Florida, twenty-six were found to be infected with hookworms. Two species have thus far been identified, *Ancylostoma braziliense* de Faria 1910 and *Ancylostoma caninum.*

Detection and Incidence of Human Intestinal Protozoa by the Sigmoidoscope. Justin M. Andrews, School of Hygiene and Public Health, Johns Hopkins University.

Lower sigmoid contents have been obtained from 253 patients sigmoidoscopically. Of this number, 210 supplied defecated specimens as well. These specimens were examined for protozoan parasites microscopically and culturally (for flagellates), and yielded, in the group which had not partaken of barium sulphate for at least six days prior to the sigmoidoscopic examination, an incidence of all organisms of 13.7% in defecated specimens as compared with 46.3% from sigmoid contents. In the group where barium sulphate had been ingested within six days of examination, a total incidence of 8.7% from defecated specimens as compared with 20.9% from sigmoidoscopic specimens was determined. The combined barium and non-barium groups revealed a total incidence of 10.5% from defecated specimens and 31.9% from the sigmoid. There appeared to be no uniform association of particular organisms with particular symptoms. The serum-saline-citrate

method of flagellate culture was found to be a useful supplement to smear examination for Trichmonas and Chilomastix.

The Excretory System of *Anisakis simplex*. Justus F. Müller, University of Illinois.

The minute structure of the band-like, unicellular, excretory gland of *Anisakis simplex* has been studied and described. The minutely ramifying side branches of the main duct are found to terminate abruptly in small interconnecting chambers, of a diameter of 0.003 mm., hollowed directly out of the cytoplasm, and bearing a shrunken coagulum. These chambers are designated as secretory passages, and the familiar central duct with its branches as drainage tubules. The drainage tubules at the point of their junction with the above chambers are 0.001 mm. in diameter. The peculiar nucleus of the gland has been studied and certain unusual modifications described. At either end of the nucleus in the regions of the anterior and posterior constrictions respectively, has been found a zone of large spherules staining orange in Mallory's double stain. These spherules are from 0.0005-0.008 mm. in diameter, and are of a peculiar type found nowhere else in the worm. Contrary to the suggestion of Goldschmidt (1906), it seems difficult to homologize this excretory system with other types known in nematodes.

The Life History of a New Forked-Tailed Cercaria. Alvin R. Cahn, University of Illinois.

The cercariae are brilliantly yellow and very active. They emerge from the anus of the snail host, *Pleurocera acuta*, as an anchor-shaped cercaria, bearing an external distome at the anterior end. Within a few minutes after hatching a marked swelling in the body of the cercaria is noticed. This is followed by a very rapid withdrawing of the distome into the internal cavity of the cercaria. The emergence of the cercaria is normally between 6 and 9 o'clock in the evening, and the activity, which consists of "flapping" to the surface, continues with gradually decreasing vigor, for about ten hours. This activity is reversible upon contact with any object or with the surface film, not unlike that of the mosquito wriggler. As the snail host is a shallow water inhabitant, the cercariae have a vertical distance of from 4 inches to 18 inches to travel. The light color, together with their conspicuous activity, make the cercariae most attractive objects, and they are eagerly devoured by the smaller fish of the shallow water, insect larvae and crayfish. If not eaten before their vitality decreases sufficiently to prevent their rising from the bottom, they are buried and lost. The adults have been found in the young of fish belonging to the family Centrarchidae, as it is only the young that are frequenters of the very shallow water during the night. The determining factor is found to be the pavement-like pharyngeal teeth of this group, which rupture the wall of the cercaria, thus freeing the distome. On being freed, the distome attaches itself at once, and hence is found only in the esophagus. The identity of the adult in relation to the cercaria in question has been proven by experimental infection. Experiments intended to alter the nocturnal hatching rhythm of the cercaria have failed; changes in temperature, light and H-ion concentration do not alter the rhythm. It is of interest to note that the snail *P. acuta* is distributed throughout the Mississippi valley, but that infected individuals have been found only within a very circumscribed area in the Oconomowoc river at the outlet of Oconomowoc lake. Here 96% of the individuals are heavily infected, 41 redia each containing 2 cercariae, having been removed from the intestine of a single snail.

Nematodes belonging to the Genus Setaria parasitic in the eyes of Horses. Benjamin Schwartz, Bureau of Animal Industry, U. S. Department of Agriculture.

Three species of nematodes belonging to the genus Setaria occur in the anterior chamber of the eyes of horses. Railliet and Henry (1911) reported that all specimens of Setaria from the eyes of horses in their collection proved to be *Setaria labiato-papillosa*. The present writer found, however, two cases in the United States in which the parasite proved to be *Setaria equina* and a third case from Indo-China in which the parasite is *Setaria digitata*. The validity of the latter species is somewhat doubtful and may prove upon further investigation to be identical with *Setaria labiato-papillosa*.

A Note on Cestode Parasites of Birds. Edwin Linton, University of Pennsylvania.

A report on cestode parasites of birds to be published in the Bulletin of the U. S. National Museum, contains descriptions of 34 species belonging to 14 genera, from 28 specific hosts. The report is based chiefly on material collected at Woods Hole, Mass., by the late Vinal N. Edwards, from marine fish-eating birds. Collections were made throughout the year and over a series of years. It was found necessary to make one new genus, and nine species were described as new. Hope had been entertained that this material from fish-eating birds might throw some light on the life history of some of the cestode parasites of fishes, but no linkages in life histories were discovered. Perhaps the most interesting species in the collection is an example of Fuhrmann's separate-sexed genus Dioecocestus. Fuhrmann states that two strobiles and no more, one male and the other female, are always found together in the same host. Mr. Edward's collections tend to confirm this observation of Professor Fuhrmann's.

A New Intermediate Host of *Proteocephalus pearsei* La Rue. Ralph V. Bangham, College of Wooster.

Epischura lacustris and *Cyclops prasinus* have already been reported as hosts for the plerocertoid stage of this cestode. (Ohio Journal of Science, 25:255-270, Nov., 1925).) While studying the food contents of the stomach of a 38 mm. small-mouth bass in 1926 a cladoceron was found with the plerocercoid alive in its body cavity. The same young fish had three of these larval cestodes in the stomach which had already emerged. The cladoceron bearing the plerocercoid was *Eurycercus lamellatus* (O. F. Müller).

Nematodes of Pathological Significance found in Economically Important Birds in North America. Eloise B. Cram, Bureau of Animal Industry, U. S. Department of Agriculture.

During the past year and a half the writer has had an opportunity of studying diseases caused by parasitic nematodes in economically important birds; these diseases had been previously unknown, or have been of infrequent occurrence in North America. They include infestations of the esophagus, including crop of chickens and turkeys with *Capillaria annulata;* of the gizzard of both domestic and wild geese with *Amidostomum anseris;* of the proventriculus of carrier pigeons with *Dispharynx spiralis;* of the small intestine of domestic pigeons with *Ornithostrongylus quadriradiatus;* of the intestines of quail with *Trichostrongylus pergracilis;* of the proventriculus and gizzard of wild ducks with *Echinuria uncinata* and of wild geese with a new species of Echinuria. In all of these instances the nematodes were present in large numbers and the damage done by them evident, either in clear cut pathological changes of the infested parts or in marked clinical symptoms of the bird, or both.

Artificial Hatching of the Eggs of the Thorny-headed Worm of Hogs. H. W. Manter, University of Nebraska.

Eggs of *Macracanthorhynchus hirudinaceus* removed from worms show many hatchings when soaked in water, allowed to dry, and then re-immersed in water. All these steps are necessary. The hatching seems to be due to absorption of water. The hatched larva immediately swells to several times its former size within the egg. The larva lives for a short time and undergoes characteristic movements. Eggs obtained from feces of hogs could not be made to hatch artificially. Eggs from worms fed to large grubworms produced extremely heavy infections, thousands of acanthocephalan larvae being obtained from single grubworm (*Strategus julianus* Burm.). Eggs showed no indication of hatching within earthworms.

The Effects of Changes in the Sugar Content of the Blood on Host-Parasite Relations in Bird Malaria. Mary Stuart MacDougall, Agnes Scott College.

Previous studies have shown that the course of infections in canaries with bird malaria can be modified by feeding sugar to the birds, thus presumably increasing the sugar content of the blood, and by injecting insulin into them, thus decreasing the sugar content. This work has been continued and careful

measurements made which show that changes in the sugar content of the blood actually occur, and that an increase renders the blood stream more favorable, and a decrease less favorable, as a habitat for the malarial organisms. These results indicate that relapses in malaria may be due to such changes and thus help us to understand the mechanism of one of the most puzzling phenomena concerned in host-parasite relations in malaria.

A Coccidian Parasite of the Flour Beetle, *Tribolium confusum* and *T. ferrugineum*. Wm. A. Riley and Laurence Krogh, University of Minnesota.

In the course of work on the histology of the larvae of Tribolium peculiar cells were noted which proved to be stages in the development of an undescribed coccidian. It was found in larvae from five out of seven cultures examined. In certain cultures as many as 70 per cent of the older larvae were infected. One culture which at first showed only 50 per cent infection had, after six months, as high as 99 per cent. The parasite is closely related to *Adelina mesnili* (Perez, 1899). It has not been found in the pupae or in the adult Tribolium. That it is of considerable economic importance is shown by the ease with which it is transmitted and by the high percentage of infection when present.

Zone Phenomena in Vivo Trypanolysis. William H. Taliaferro and Thurston L. Johnson, University of Chicago.

Immune sera from sheep, rabbits and guinea-pigs, infected with *Trypanosoma equinum*, will produce an artificial crisis (by Trypanolysis) in mice which have been infected with the same strain. When, however, a series of mice, on about the third day of their infection, are given immune serum over a long range of doses, trypanolytic crises will sometimes be brought about by moderate sized doses and not by larger doses. Furthermore, zones of lysis and of inhibition of lysis may recur several times in a single series. In such cases, wherever trypanolysis occurs, the length of life of the mice is approximately the same whether it has been caused by a large or small dose of serum. Moreover, mice in which lysis is inhibited, even though they may have received enormous doses of serum, die at about the same time as control mice which did not receive any serum. Hence the therapeutic value of the immune serum, as measured by the survival time of the mice, is dependent upon whether lysis occurs or not and not upon the size of the dose of serum. By isolating single-celled strains of trypanosomes, it has been found that animals infected with some strains yield an immune serum that gives "zones of inhibition of lysis" whereas animals infected with other strains yield sera that never show such zones.

The Thermal Death Point of Protozoan and Metazoan Parasites and the Application of a new Method for the Removal of Intestinal Parasites and the Treatment of Other Affections of the Intestine. Damaso de Rivas, Medical School, University of Pennsylvania.

The method consists in the application of warm physiological salt solution into the intestine, and is based on the principle, as determined by experiments made *in vitro* and *in vivo* that protozoan and metazoan parasites are extremely susceptible to the action of heat. These parasites are readily killed at 45°C. in 5 to 10 minutes, and in a much shorter time at 47°C. Histopathological studies made on animals and clinical observations in man, have demonstrated that these temperatures, when cautiously applied, do not cause any appreciable symptoms in the host. The method is very efficient and the technique offers no special encumbrance in its application. It can be used for the removal of intestinal parasites from the large and small intestine such as hookworm, oxyuris, tapeworm and round worms, etc., as well as for the treatment of amoebic dysentery, simple mucous colitis, intestinal intoxication, acute trichinosis, acute poisoning and other affections of the large and small intestine.

Behavior Studies on Larval Trematodes. Harry M. Miller, Jr., Washington University, St. Louis.

The behavior of five species of larval trematodes from *Cerithium literatum* was studied at the Carnegie Marine Laboratory at the Dry Tortugas. Four per cent of 3,797 host individuals examined were infested. The swimming behavior

and pigmentation of each cercaria is so distinctive that examination with the naked eye is sufficient to determine which species is present. Three of them exhibited what are probably geonegative reactions; in one species, Cercaria E, the reaction is especially marked, and has been shown experimentally not to be due to a CO_2 or O_2 gradient. This species remains strongly geonegative for several hours after emergence from host tissue, and after it becomes geopositive exhibits what is probably a photonegative response. During its geonegative period the rate of upward swimming of Cercaria E was determined. Three larvae react definitely to light or to changes of intensity. The strongly photopositive reaction of Cercaria F was studied; this species is geopositive in the dark. The responses of two cercariae, D and F, to changes in light intensity were studied and a preliminary determination of the threshold of stimulation for Cercaria P was carried out. Observations on the longevity of the different larvae under laboratory conditions were made; and the resistance of the strongly geonegative species, Cercaria E, to dilution with fresh water was studied. Some evidence of a diurnal rhythm of activity was found in several larvae.

A Standardized Method of Treating Tapeworm Infestations in Man with the Idea of Recovering the Head. Thomas B. Magath and Philip W. Brown, Mayo Clinic.

Because heads are so rarely found in general practice following treatment of tapeworms, it is well to have a standardized form of treatment. Such a one has been worked out by the authors, which if adhered to will result in the recovery of practically every head harbored by the patient. The steps may be summarized as (1) preparation of the patient, (2) adminstration of the anthelminthic and (3) the search for and recovery of the head.

The Value of Cultural Methods in Surveys for Parasitic Amoebae of Man. Charles F. Craig, Lt. Colonel, M. C., U. S. Army, and J. H. St. John, Captain, M.C., U. S. Army, Army Medical School, Washington, D. C.

This contribution embodies the results obtained from a survey of 71 individuals, members of the Officers' Class of the Army Medical School, for intestinal amoebae. The survey was undertaken to secure knowledge regarding the prevalence of infection with intestinal amoebae, and to determine the relative value of the Locke-Egg-Serum medium of Boeck and Drbohlav, and more simple media devised by Craig. This survey in which 71 individuals were examined, demonstrates that cultural methods are superior, for diagnostic purposes, to either the sedimentation method or the direct examination of the feces, when only one microscopical preparation is examined. The results obtained in the survey with cultural methods showed that 39 of the 71 individuals examined were infested with one or more species of amoebae, or 54.92 per cent. *Endamoeba histolytica* was present in 11, or 15.49 per cent, *Endamoeba coli* in 21, or 29.57 per cent; *Endamoeba nana* in 9, or 12.67 per cent; and *Iodamoeba williamsi* in 4, or 5.63 per cent. In eight individuals *Endamoeba histolytica* occurred alone; in sixteen, *Endamoeba coli* occurred alone; in five, *Endamoeba nana* occurred alone; and in two, *Iodamoeba williamsi* occurred alone. In eight individuals there were mixed infestations. The survey demonstrates that the Locke-Serum medium gave a larger percentage of positive results than did any of the other methods employed, and that neither coagulated egg or a solid substratum of any kind is necessary for success in surveys for the intestinal amoeba. The successful results with a medium composed of seven parts of normal-salt solution and one part of inactivated human blood serum demonstrate that none of the chemicals in either Locke's or Ringer's solution, with the exception of sodium chloride, were essential in the media used in the survey and that the continued cultivation of *Endamoeba histolytica* over an indefinite period of time is possible in this simple medium, as shown previously by Craig.

A Comparative Study on the Longevity of *Opalina obtrigonoidea* in Various Media. Mary E. Larson and Fred W. Allen, Jr., University of Kansas.

Opalina obtrigoniodea, a common ciliate protozoan parasite found in the cloaca of the leopard frog (*Rana pipiens*) can be successfully cultured in a number of different media.

The more common laboratory media such as physiological salt, Ringer's solution and Locke's solution were tried first. It was found that if a piece of the cloacal wall was left in the culture the parasites lived longer and appeared more nearly normal. In Locke's solution the culture remained fairly good up to the fourth day. In almost all of these cultures the Opalina divided at least once and the daughter Opalina appeared fairly normal. After testing out the above mentioned solutions, we tried Cleveland's serum-saline-citrate solution and Pütter's sodium chloride-sodium and potassium tartrate solution and various modifications of these and other media with considerable success. None of these media were made oxygen free. In several cultures in the modified Locke's solution and also in Pütter's solution, the organisms grew and divided from 14 to 21 days. Transfers from these were made every second day.

Excystation in the Rat of *Giardia lamblia* from Man. R. W. Hegner, School of Hygiene and Public Health, Johns Hopkins University.

Washed cysts were injected into the stomach of laboratory and wild rats and the animals killed one, two and four hours later. During these periods some of the material injected passes into and through the small intestine and in some cases enters the cecum. Examinations of the contents from various regions of the small intestine indicate that a short sojourn in the stomach is not fatal to the cysts. Cysts, apparently viable, were also recovered throughout the small intestine and cecum. Cysts were found in the small intestine within which the organism was actively moving about—a condition never observed by us in cysts outside of the body. Trophozoites were recovered from the small intestine, usually about 30 cm. posterior to the stomach, that correspond morphologically to *Giardia lamblia*. The animals used were not infected with giardias before the experiments were begun so far as could be determined, hence, although the trophozoites recovered may be those of previous infection with *Giardia simoni*, which resembles *G. lamblia* of man, it seems probable that they are excysted forms of the latter.

The Report of a nearly pure *Ancylostoma duodenale* Infestation in Native South American Indians and a Discussion of its Ethnological Significance. Fred L. Soper, International Health Board.

Seventy Lengua Indians, living under semi-civilized conditions in the Paraguayan Chaco almost without contact with the outside world, have an Ancylostoma/Necator ratio of 13 to 1. The comparison of this ratio with the much lower Ancylostoma/Necator ratios reported for civilized Paraguayans (1 to 14), for Brazilian Indians (1 to 57), and native Brazilians (1 to 194) suggests that the infestation has not been entirely introduced from without since the discovery of America by Columbus, and that its character may help establish the origin of the American Indian.

A New Species of Strigea from the Herring Gull, *Larus argentatus* (Pont.), with Remarks on the Function of the Hold-Fast Organ. George R. La Rue, University of Michigan.

An apparently new species of Strigea occurring in the bursa fabricii of young herring gulls, *Larus argentatus* (Pont.) is described. This parasite when abundant, causes emaciation and paralysis, and finally the death of the host. In the genus Strigea the hold-fast organ, functioning in conjunction with the beaker-shaped fore-body, forms a powerful organ for attachment. Host epithelial tissues in contact with the hold-fast organ are corroded, thus bringing the underlying vascular layer into direct contact with the surfaces of the hold-fast organ and the fore-body. Corrosion may be caused by enzymes secreted by gland cells within the hold-fast organ. The intestinal branches are always empty and the oral sucker is so placed that materials cannot readily be ingested from the lumen of the alimentary tract of the host. Hence, it seems that the hold-fast organ, together with the inner surfaces of the fore-body, may serve as important organs for the nutrition of the parasite. A comparison of the hold-fast organs of other members of the family is made with respect to structure and probable function.

Egg-Worm Correlations in *Fasciolopsis buski* cases, with Additional Data on the Distribution of this Parasite in China. N. R. Stoll, W. W. Cort, and W. S. Kwei, School of Hygiene and Public Health, Johns Hopkins University.

A series of carefully studied cases parasitized with the large intestinal fluke in Shaoshing, Chekiang province, China, indicate that individual flukes lay on an average of about 25,000 eggs per day. Incidence data obtained during the work of the China Hookworm Commission indicate apparently endemic cases of *Fasciolopsis buski* in Northern Shantung, Hupeh, and Southern Kiangsu provinces. The latter "borders" the heavily infested Shaoshing area on the north.

Some Notes on the Genus Leucochloridium. Allen McIntosh, University of Minnesota.

Immature stages of the trematode genus Leucochloridium occur as multicolored banded sporocysts in the tentacles of snails. Studied especially in Europe and recently in this country by Magath they are known chiefly in this larval form. To the present, no adult flukes of the genus have been reported from North America. This paper considers specimens from five species of our native birds and includes a table to the species of the genus.

LUNCHEON AND BUSINESS MEETING

The luncheon of the Society which was held Wednesday noon, December 29, 1926, at the Hotel Pennsylvania was attended by seventy-nine members of the Society and guests. After luncheon the annual business meeting was held, the minutes of which were as follows:

The meeting was called to order by the President, Dr. Charles W. Stiles; he introduced to the Society the following officers and members of the Council who were seated at the speaker's table, viz., W. A. Smillie, R. W. Hegner, H. B. Ward, W. W. Cort, W. A. Riley, and E. E. Tyzzer. The President then introduced Dr. G. H. F. Nuttall, the guest of the Society at the luncheon, who made a short speech. The minutes of the second annual business meeting at Kansas City were read and approved.

A preliminary report of the Treasurer for 1926 was read.

The report of the Secretary showing a total membership of 392 was approved.

The following recommendation of the Council in regard to the China Branch was adopted:

a. The formal recognition by the American Society of Parasitologists of the group in China as the China Branch of the Society.

b. The election of the Chairman of that branch (at present Dr. E. C. Faust) as a second Vice-President of the Society and a member of its Council.

c. The recognition by the parent society of the autonomy of the branch in any matters which relate only to its local organization and work. The individuals who make up the China Branch conform to the constitution of the American Society of Parasitologists in methods of election to the Society, payment of dues, etc.

The following By-law was recommended by the Council and adopted:

Members of the Society who fail to pay the dues for two years may be dropped from the roll by vote of the Council, provided they have been given due notification by the Treasurer of the rules of the Society in regard to delinquent dues. Such individuals may be reinstated at any later time by vote of the Council.

A report of progress was made by the committee on the Teaching of Parasitology, by F. D. Barker, Chairman. It was stated that the committee had prepared a questionnaire which would be sent to the members of the Society early in 1927. The report was approved and the committee continued.

A report of progress was made by the Chairman of the Committee on Publication, Dr. H. B. Ward. The report was approved and the committee continued.

The following report of the committee on Nomenclature was read by the Secretary in the absence of the Chairman, Dr. M. C. Hall. During the consideration of this report the chair was taken by Dr. H. B. Ward. The report of this committee was adopted and ordered printed in the minutes of the meeting. The committee was not continued.

REPORT OF THE COMMITTEE OF NOMENCLATURE

As regards the proposals submitted by Dr. Poche, the American Society of Parasitologists takes the position indicated in the following paragraphs:

1. Dr. Poche proposes to amend Article 25 of the International Rules of Nomenclature, which reads as follows: "The valid name of a genus or species can be only that name under which it was first designated on the condition: *a*. That this name was published and accompanied by an indication, or a definition, or a description; and *b*. That the author has applied the principles of binary nomenclature." This is known as the law of priority. Dr. Poche's emendation is in the nature of an enlargement of proviso *b* of Article 25, which reads "That the author has applied the principles of binary nomenclature." The emendation would throw out of court all "*publications* in which the author offends against the principles of binary nomenclature."

In comment we would note that in general the recommendation is more or less in accord with the code, and that the intent is to lessen confusion in nomenclature resulting from unfortunate substitutions of unfamiliar names for well established ones. The recommendation differs from the code in that the code recognizes names conforming to *binary* nomenclature even though species are not cited as binomials. Unfortunately certain important publications antedate zoological codes formally adopted by zoological societies and certain authors proposed some generic names which may be recognized under the code, although they used no name or polynomial names for the species. There would be, undoubtedly, some advantages in throwing out of court publications which are not consistently binomial in nature, but, as is usually the case, there would also be disadvantages. While Prof. Poche's intent is to lessen confusion in well established names, the effect would be to increase it in certain cases as it would involve the rejection of some names now in common use and would change the dates of some other commonly used names.

In view of the complications and the controversial character of the subject, this society would prefer to leave this subject to the Commission on Zoological Nomenclature as a body of experts competent to deal with it rather than to see it subjected to an open vote by the Congress of Zoology, since many members of that Congress will have but little interest in or knowledge of the subjects of taxonomy and nomenclature and hence can not speak with authority or vote on adequate information on this subject.

2. Dr. Poche proposes to restrict Article 30g of the code, which reads as follows: "If an author, in publishing a genus with more than one valid species, fails to designate (see *a*) or to indicate (see *b*, *d*) its type, any subsequent author may select the type, and such designation is not subject to change. (Type by subsequent designation.)" Dr. Poche's emendation provides an amendment (as a *rule*) to Article 30e. [The following species are excluded from consideration in determining the types of genera] to read as follows: "(d) Species which have already been removed ("eliminated") from the genus." He adds detailed provision as to what does or does not constitute "elimination."

This topic is dealt with in the code as *Recommendation* k of Article 30, as follows: "If some of the original species have later been classified in other genera, preference should be shown to the species still remaining in the original genus. (Type by elimination.)" In effect then Dr. Poche's proposal would make a *rule* of the present recommendation; further it would define the acts which do and do not constitute "elimination," a matter which is subject to pronounced differences of opinions.

Again it does not appear to this society advisable to adopt this detailed rule proposed by Dr. Poche. At present the first proposal of a type species is accepted, whether this type was selected under the first species rule or the elimination rule. This policy was adopted as a satisfactory and workable compromise between the "first species" advocates and the "elimination" advocates and has been in force since 1907. The proposed rule abrogates this compromise and reopens the controversy which both sides have for many years considered was a closed issue.

There was formerly much controversy on this point and we prefer the expert consideration of an international commission to the necessarily casual or biased action of a congress largely composed of persons not specifically interested in nomenclature.

3. Finally Dr. Poche proposes that all suggested modifications of the code which may be approved by a *majority* of the International Commission are to be submitted to the congress for a vote. In this proposal we can not concur. The practice of requiring a unanimous vote of the commission as a preliminary to all recommendations of the commission to be brought before the congress is a sound one and in accordance with good scientific and deliberative procedure. This policy has, in fact, been an important factor in gaining adherents to the International Code.

In the first place the commission is more truly international in character and better balanced in this respect than is the congress, since the commission is selected on this basis, whereas the congress will always have a preponderate membership from the country in which it meets and from adjoining countries. The commission changes its personnel by slight replacements and maintains some uniformity and continuity of information, whereas the congress changes its personnel very materially at each meeting.

In the second place the commission is selected from the comparatively few men familiar with the details of the code and the many intricacies of its application and hence has special and expert knowledge of the topics involved, whereas many of the members of the congress have no such knowledge or familiarity.

In the third place, the existence of a division of opinion in the commission would certainly be indicative of a division of opinion on the floor of the congress and it appears to us a useless waste of the time of the congress to indulge in controversies on nomenclature under conditions which are unfavorable for careful consideration of the topics involved and provocative of unpleasantness. Rules of nomenclature passed by a mere majority of the congress, especially on points where the lines of division in practice might follow lines of nationality rather closely, as is sometimes the case, are likely to be violated by a large minority and to be changed whenever the congress meets in a country where the practice is unfavorable to the rule previously passed. Such procedures could only result in confusion in nomenclature, instead of the clarity and certainty which Dr. Poche and zoologists in general wish.

In the fourth place Dr. Poche's proposition does not safeguard the interest of the minority, and is therefore open to serious objection.

We therefore recommend that Dr. Poche's proposals be referred to the international commission for consideration and decision.

MAURICE C. HALL.
BENJAMIN SCHWARTZ.

The nominating committee consisting of H. B. Ward, chairman, R. W. Hegner, and J. W. Scott, made the following nominations for 1927:

President, R. P. Strong.

Vice-President, E. Linton.

Secretary-Treasurer, W. W. Cort.

Members of the Council for four years, F. D. Barker, J. H. St. John.

The report of the committee was accepted and a unanimous ballot was cast by the secretary for the names proposed.

A resolution of thanks to the Secretary-Treasurer for his work for the year was passed. The meeting then adjourned.

W. W. CORT, *Secretary.*

NEW HUMAN PARASITES

Schistosoma incognita Chandler 1926. This species is based on eggs of distinctive size and shape found in two human stools in Bengal. The adult parasite is unknown. The eggs are roughly like *S. japonicum*, being 95 to 100μ by 42 to 50μ, and bear a small subterminal spine (7 to 8μ long) slightly offset from the pole toward one side, on which the egg is flattened. While the above characters seem to be specific, further study is desirable before passing judgment of the exact status of the new form. (Indian J. M. Res., 14: 179, pl. V; July, 1926.)

BOOK REVIEWS

Researches on Hookworm in China. By W. W. Cort, J. B. Grant, N. R. Stoll and other collaborators. Amer. Jour. of Hyg. Monographic series, No. 7, Oct., 1926; 398 pp., numerous plates and textfigures.

The work of the China Hookworm Commission from June, 1923, to November, 1924, is presented in this book, which "is dedicated to the memory of Samuel Taylor Darling, whose researches ushered in a new era in the scientific control of hookworm disease." The work is divided into fifteen chapters, dealing with the methods of attack, the geography of China in relation to the hookworm problem, information obtained from literature and from questionnaires, the significance of hookworm infestation in North China, and separately five other districts, its relation to rice cultivation, to cotton cultivation, and experimentally to the cultivation of vegetables, the economic value of nightsoil in China, the viability of hookworm eggs, and finally a carefully thought out summary of results. It would be impossible within the limits of space to cover the many important studies made and the valuable results secured by these workers. One can only express surprise that so much could have been done at the time at their command. The numerous illustrations add clearness and carrying power to the text. No one interested in this problem can afford to neglect reading the original.

Acta Leidensia Edita Cura et Sumptibus Scholae Medicinae Tropicae. Vol. I. (Inst. v. Trop. Geneeskunde, Leiden, Holland); 336 pp., many textfigures and plates.

All students of parasitology must be greatly interested in the development of the Leiden Institute of Tropical Medicine. This interest will be heightened by the perusal of the first volume of contributions from the Institute; this has appeared in the form of a finely printed book of 325 pages with numerous plates and textfigures. The book opens with a description of the Institute, which occupies a beautiful ancient mansion that after having been devoted for many years to other state purposes was in 1920 assigned by the government to the work of an institute of tropical medicine and completely rebuilt to suit its purposes. The Director of the Institute, Dr. P. C. Flu, is Professor of Tropical Hygiene at the University of Leiden, and the movement is fortunate in having the active support of the Society of Tropical Medicine at Rotterdam. All of those concerned may be congratulated, not only upon the admirable quarters and the valuable practical work conducted there, but also on the researches which are presented in this first volume of the proceedings. Many of them are bacteriological. Among those of immediate interest to students of animal parasites are papers on anemia in hookworm disease, on Anopheles, on the life history of trematodes, on various nematodes, and on Rickettsia.